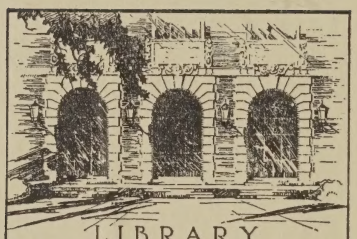

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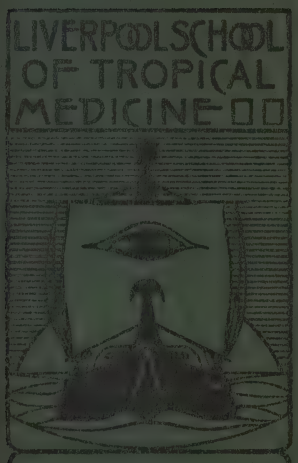

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1921	Stewart, Robert Bell
1921	Thomson, Marion
1922	Bhatia, Jagat Ram
1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John
1922	Rieley, Stanley Desmond
1922	Rutherford, Gladys
1922	Stewart, Quinton
1923	Basu, Dhirendranath
1923	Cruickshank, John Cecil
1923	Doherty, Winifred Irene
1923	Elsohn, John
1923	Raja, Rojaporum
1923	White, Charles Francis

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX. pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal o Pathometry*, Vol. I, pp. 1-20.

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A CASE OF CREEPING ERUPTION IN A EUROPEAN IN THE GOLD COAST

BY

J. F. CORSON

(*Received for publication* 14 *November*, 1922)

PLATE I

This skin affection is stated by Crocker (1903) and by Castellani and Chalmers (1919) to have been first described by Robert Lee in 1875; according to Roubaud (1914) the disease was observed in Norway by Hoegh in 1869, while Abraham, in a review of a paper by Knowles (1916), said that cases were recorded in Edinburgh 'more than sixty years ago,' i.e., before 1856.

When a cause has been found, it has usually been a larva of a fly of the family *Oestridae*, particularly *Gastrophilus* and *Hypoderma*. Castellani and Chalmers (*loc. cit.*) state that larvae of *G. haemorrhoidalis* and *G. nasalis*, of *Oestromyia satyrus* and of *H. bovis* and *H. lineata* have been found. Looss said that larvae of *Ancylostoma duodenale* in their passage through the skin could cause it. Sakurane (1917) found a *Ligula* parasite in a swelling of the skin, and suggested that the parasite of creeping eruption is of this nature. Ikegami (1919) removed from a case of this disease a young worm, said to have been probably *Echinorhynchus sphaerocephalus*, but a structure in it like an alimentary canal suggested *Gnathostoma*. Tamura (1919) removed a male *Gnathostoma* resembling *G. siamense*.

The disease is reported to have occurred in Ireland, Scotland, the Shetland Isles, Norway, Sweden, Denmark, Russia and Siberia, Bulgaria, Arabia, Sumatra, China, Japan, the United States of America, Brazil and West Africa (Senegal, Sierra Leone, Liberia, Togoland, Nigeria and the Cameroons).

The form of the disease occurring in Senegal, called locally Oerbiss or Larbish, and for which no cause has been found, is considered by Roubaud to be of different aetiology from cases due to myiasis.

The following case showed a close resemblance clinically to the description of Oerbiss given by Roubaud.

Mr. G., British, living at Seccondee, Gold Coast, noticed, about the 18th of June, 1922, a small itching spot on the ball of the left thumb, and thought that it was probably due to a bite of some insect. A few days afterwards he noticed that the spot had become a line, and by the 26th of June there was a curved, raised, blister-like line about three-quarter inch long and one-sixteenth inch in diameter. Itching and a burning sensation were considerable, especially at night. On the 6th of July the appearance was as shown in Plate I. From then onwards until the beginning of October, when opportunities of observing the case ceased, the track progressed irregularly and intermittently round and along the thumb to near the tip. No parasite was found; microscopic examinations of serum and blood taken from various parts of the track and attempts at culture in broth and on agar and blood serum were without result.

No serious attempt to cure the disease was made; an ointment of sulphur and ammoniated mercury was used by the patient, who also opened the tracks from time to time and rubbed in tincture of iodine with apparent temporary benefit.

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EXPLANATION OF PLATE I

Case of Creeping Eruption.

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DEPTH, AND THE LARVAE AND PUPAE OF *STEGOMYIA FASCIATA*, F.

BY

J. W. S. MACFIE

(*Received for publication* 14 *November*, 1922)

The larvae of *Stegomyia fasciata*, as is well known, are usually found in small collections of water in domestic utensils, old tins, rot-holes in trees, calabashes, rock pools, etc.; they are to a large extent bottom- and side-feeders, and are capable of remaining, and in fact frequently do remain, completely submerged for very considerable lengths of time. In many tropical countries where *S. fasciata* is prevalent, water is stored in tanks of some size and depth. Even if efficiently screened, larvae may gain access to these tanks by being washed in with rainwater as eggs or young larvae, and it is a matter of some interest to know if, supposing they were introduced in this way, the larvae of *S. fasciata* would be able to thrive or would be likely to escape by being drawn off with water from a tap situated near the bottom.

Iyengar (1920) observed at Calcutta that larvae of *S. fasciata*, which are found there in almost all domestic situations in which larvae of *Anopheles stephensi* are found, are, however, rarely encountered with them in wells. He accounts for this difference in habit by the fact that whereas the larvae of *A. stephensi* are provided with hooks at the ends of the dorsal hairs on the ninth abdominal segment by means of which they cling to the sides, the larvae of *S. fasciata* lack these hooks, and, therefore, he assumes, were they to frequent wells would have to go to the bottom if the water was disturbed. 'It is likely,' he thinks, 'that mosquito larvae, being air-breathing organisms, cannot ordinarily stand much pressure at the depth of a well. Stegomyia is a bottom- and side-feeder; therefore it has to go to the bottom of its breeding-place, unlike *A. stephensi*, which feeds on the surface. These facts explain why *Stegomyia* has rarely been found in waters which are over three feet deep.'

These statements set us wondering if it was, indeed, the case that larvae of *S. fasciata* could not withstand the pressure of more than about three feet of water, and if disturbed must inevitably go to the bottom. The following experiments were carried out to ascertain the facts.

The apparatus used consisted simply of a wide-mouthed bottle with sloping shoulders, connected by a stout piece of rubber tubing with a length of wide-bore glass tubing. The tube and the bottle were set up vertically, the one above the other, and securely clamped. When required, additional lengths of tubing were added at the top with short rubber connexions.

In such a system larvae of *S. fasciata* lived apparently at ease, and after a day or two congregated at the top, mostly in the first foot, a few in the second, and only stray individuals at greater depths. The successive stages observed in an actual experiment are shown in the Table. As will be seen, the larvae, which at first were

Day	1	2	3	4	5	6
1st foot	+	+	++	++ (Mostly at the top)	++ (Nearly all at the top)	++ (Nearly all at the top)
2nd foot	+	+	++	+−	5	1
3rd foot	+	+	+−	o	1	o
4th foot	+	+−−	1	o	o	o
5th foot	+	+−−	o	o	o	o
6th foot	+	+−−	o	o	o	o
7th foot	+	+	o	o	o	o

++ = many Larvae. + = several Larvae. +− = few Larvae.
+−− = very few Larvae. 1, 5, = one, five Larvae.

distributed throughout the tube, collected rather slowly at the top, so that after three days almost all of them were in the first foot of the column of water, and the majority at any particular moment actually at the surface. During this process of settling the habits of the larvae changed, bottom feeding being discontinued.

If then the tube was shaken or tapped, the larvae left the surface and wriggled down in the usual manner. They did not, however,

sink to the bottom; indeed, most of them descended only a few inches and very few more than one foot. Their descent was not passive, but was effected by active wriggling movements, and when these ceased they immediately began to float upwards towards the surface. Under ordinary circumstances, if disturbed the larvae wriggled downwards a few inches, ceased wriggling and floated upwards a short distance, and then recommenced active wriggling, this time towards the surface. They did not attempt to cling to the side of the tube. It is clear, therefore, that the larvae of *S. fasciata* when disturbed do not necessarily go to the bottom.

As the result of a single tap on the tube, it occasionally happened that one or two larvae descended to greater depths, such as two and a half feet or even three and a half feet. Larvae were also sometimes observed to descend voluntarily as much as five feet, and once one was found browsing on the side of the tube at a depth of 6 feet. By repeated tapping on the tube the larvae, could be urged to descend even deeper, eight feet at least. They did not appear to be at all incommoded by the pressure of the column of water, and when the tapping ceased wriggled back to the surface. Sometimes they rested on the bottom for a short time before starting the upward journey. It took one larva six minutes to regain the surface after descending seven feet.

In one experiment the system, consisting of the bottle and a long glass tube of wide bore of a total length of seven feet, was left standing until a copious growth of green algae had formed over the bottom, from which small bubbles of gas arose in sunlight and presumably kept the water oxygenated. In this system larvae of *S. fasciata* throve better than they did when no algae were present, and were more frequently seen at greater depths; indeed, both young and older larvae, but especially the former, were often seen browsing actually on the bottom. The pressure of the seven-foot column of water above them appeared to have no injurious effect whatsoever.

The pupae of *S. fasciata*, however, are not able to descend unharmed to such great depths as the larvae. As the result of a single tap on the tube, they usually descended only an inch or two and then floated passively back to the surface. By repeated tapping they could be induced to descend considerably further, but beyond

a certain depth (which in our experiments appeared to be about three feet to three and a half feet) they showed an unquestionable anxiety to return to the surface, ceasing to respond readily to disturbances, such as tapping or shaking, even when violently applied, descending further only very reluctantly, and sometimes refusing to move at all or actually ascending in spite of everything. In one experiment, by means of repeated tapping and shaking, a pupa was driven down to the bottom, a distance of seven feet. From this position it struggled upwards, evidently with increasing difficulty, for a distance of a little more than four feet. At about this level it managed to maintain itself for several minutes, now jerking itself up an inch or so, now sinking an inch or so, and then began to lose ground, at first slowly, then more quickly, and eventually sank to the bottom. Another pupa was similarly induced to descend five feet, but it managed to regain the surface. The inability of pupae to descend without ill-effects to such great depths as the larvae appeared to be dependent on their diminished buoyancy at such depths, which caused them to begin to sink the moment active movement was arrested. This fact should be correlated with the imperative need of pupae of access to air, for the strenuous efforts exerted in struggling upwards from an unaccustomed depth no doubt accelerated the exhaustion of the supply of air in their tracheal tubes.

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A NOTE ON THE ACTION OF LITHIUM CHLORIDE ON MOSQUITO LARVAE

BY

J. W. S. MACFIE

(*Received for publication* 14 *November*, 1922)

It is well known that larvae of certain mosquitoes, e.g., *Stegomyia fasciata*, are intolerant of common salt. Other chlorides act similarly, some of them very powerfully, as is shown in Table I,

TABLE I.

The number of hours required to kill all larvae of *Stegomyia fasciata* in various soloutions of chlorides.

Salt	Percentage of anhydrous salt which = 1·0% Cl	Cl 1·0%	Cl 0·75%	Cl 0·5%	Cl 0·25%
$ZnCl_2$	1·92	3 hours	3 hours	7 hours	7 hours
$BaCl_2$	2·93	4 hours	5 hours	<24 hours	<24 hours
LiCl	1·21	5 hours	7 hours	>7 hours	<24 hours
NaCl	1·65	6 hours	<24 hours	>48 hours	>72 hours
$CaCl_2$	1·56	6 hours	>24 hours	>48 hours	>72 hours
$MgCl_2$	1·34	<24 hours	>24 hours	>48 hours	>72 hours

which summarises a series of preliminary experiments on the action of these salts carried out by Mr. R. Simmons, which I am permitted by him to quote. Additional experiments were made subsequently with lithium chloride and *Stegomyia fasciata*. In one of these, five larvae and one pupa were placed in a 1·2 per cent. solution of LiCl; within four hours all the larvae were dead, but the pupa appeared to be unaffected. In three others, twenty-seven larvae were placed in a 0·3 per cent. solution of LiCl in the afternoon; all were dead by next morning, that is within sixteen or seventeen hours.

During the experiments it was noted that lithium chloride not only killed the larvae of *Stegomyia fasciata*, but also produced a peculiar effect on them, causing them to writhe about at the bottom of the jars, apparently unable to rise to the surface, and to become entangled with one another, usually by the mouth brushes. These effects were observed even in the weakest solutions used.

As lithium chloride appeared to have a very powerful effect on the larvae, further experiments were carried out to determine the limits of the injurious action.

Culex fatigans. The larvae were placed in glass jars (five in each) containing 100 c.c of the lithium chloride solution. The jars were covered with glass plates, stood on the laboratory bench, and examined morning and afternoon at about 9 a.m. and 5 p.m. The solutions used were 0·3, 0·15, 0·06, 0·03, and 0·015 LiCl per cent. The results are summarised in Table II.

TABLE II.

The effect of solutions of Lithium chloride on the larvae of *Culex fatigans*.

Day of the experiment	Percentages of LiCl in the solutions				
	0·3	0·15	0·06	0·03	0·015
1. a.m.	Experiments started				
p.m.	Three dead	All affected	One affected	No visible effect	No visible effect
2. a.m.	All dead	All dead	Three affected	No visible effect	No visible effect
p.m.	—	—	All sluggish	No visible effect	No visible effect
3. a.m.	—	—	Three almost dead	No visible effect	No visible effect
p.m.	—	—	Two just alive	No visible effect	No visible effect
4. a.m.	—	—	—	No visible effect	No visible effect
p.m.	—	—	One alive	No visible effect	No visible effect
5. a.m.	—	—	All dead	No visible effect	No visible effect
p.m.	—	—	—	No visible effect	No visible effect

Exactly similar experiments were carried out with larvae of *Stegomyia fasciata* and *Anopheles costalis*. Without entering into details, it may be said that the results also were similar, all, or practically all, the larvae dying in the 0·3 and 0·15 per cent. solutions within twenty-four hours, and in the 0·06 per cent. solution

within two or three days. In the 0·03 and 0·015 per cent. solutions the larvae, especially when young, were also affected: in an experiment with almost fully grown larvae of *S. fasciata*, for example, only two out of ten completed their development in the former solution, and two out of seven in the latter, whereas in the control jar no casualties occurred. In the case of *S. fasciata*, entanglement of the larvae by their mouth brushes and other setae was repeatedly, but not invariably, observed.

It is worthy of note that the larvae of *Mansonioides africanus*, which live attached to the roots of the water-weed *Pistia stratiotes*, do not escape the action of lithium chloride. A small plant of *Pistia stratiotes* with larvae attached to it was placed one afternoon in a jar containing 100 c.c. of a 0·3 per cent. solution. By the next morning, that is within eighteen hours, all the larvae had left the roots of the plant and were dead.

MALARIA IN CHIMPANZEES IN SIERRA LEONE

BY

S. ADLER

(*Received for publication* 15 *November*, 1922)

PLATES II AND III

Reichenow (1920) working in the Cameroons, found parasites indistinguishable from human malaria parasites in the blood of gorillas and chimpanzees. Of eight chimpanzees examined six were found to be infected, one with *Plasmodium vivax* ? forms (gametocytes only), two with *Plasmodium falciparum* forms (crescents only), one with *P. falciparum* and *P. vivax* forms, and two with *P. falciparum* and *P. vivax* forms together with *P. malariae* forms.

Reichenow found that infections were heaviest in young animals, and suggested that resistance is acquired after attacks in early life.

Blacklock and Adler (1922), of the Liverpool School of Tropical Medicine, described a parasite resembling *Plasmodium falciparum* in a chimpanzee, and forms resembling *P. vivax* and *P. malariae* also occurred, but the only form of gametocyte found was the crescent.

I have recently examined thirteen additional chimpanzees, six of which were caught near Pendembu, and six near Blama, in the Sierra Leone Protectorate, and one from an unknown locality.

Of these thirteen animals, two were found to be infected with parasites indistinguishable from *P. falciparum*. The infected cells were not enlarged or pale, and many of the delicate rings showed two bars of chromatin. In both cases crescents were found, but only after prolonged search, resembling in this respect human infections with *P. falciparum* in West Africa. Parasites resembling simple tertian or quartan forms were not found.

CASE I. Captured near Blama. The animal was emaciated and weak. A blood examination on 8th September, 1922, showed numerous rings and a few crescents. On 9th September, 1922, quinine hydrochloride, 0·5 grains, was administered intramuscularly; rings were present in the blood until 14th September, 1922, but crescents persisted until the animal's death on 2nd October, 1922.

The animal's condition showed no marked improvement after the disappearance of rings from the peripheral blood; its appetite was poor and it often passed loose stools containing a large amount of fat globules. Death occurred after an attack of enteritis, which was apparently caused by an invasion of *Oxyuris* sp., of which large numbers (all immature) were passed in the animal's stool.

Post-mortem, malaria pigment was found in the spleen, liver, and bone marrow, and crescents, in small numbers, in the bone marrow. The liver showed fatty changes. Enormous numbers of immature *Oxyuris* were found in the large intestine.

25th September, 1922. Advantage was taken of the fact that rings had not been seen in the blood for eleven days, and that crescents still persisted, to test the theory of parthenogenesis. 0·4 c.c. of the animal's blood were injected into another chimpanzee in which malaria parasites had never been found since it first came under observation on 4th September, 1922.

The injected animal was observed till the 11th November, 1922, but parasites were not found in the peripheral blood.

CASE II. Captured near Pendembu. The animal was extremely emaciated. On 12th September, 1922, rings and crescents were found, but the infection gradually disappeared without treatment, and on the 19th September, 1922, the blood became negative. The animal's condition gradually became worse, it took very little food, the stools were loose and always contained fat globules.

On 30th October, 1922, the blood examination again showed a few ring-form parasites.

The animal died on 30th October, 1922. Post-mortem pigment was found in the spleen and bone marrow, and a small number of schizonts in the spleen; no crescents were found. The liver was pale, and on section showed extreme fatty degeneration and infiltration, the majority of the liver cells being destroyed.

It is interesting to note that both animals were young (under two years). Older animals, including one old adult, were negative. This supports Reichenow's suggestion that in chimpanzees, as in natives, resistance is acquired after attacks in early life.

THE RELATIONSHIP OF MALARIA IN CHIMPANZEES TO HUMAN MALARIA IN SIERRA LEONE

Although the malaria parasite in the chimpanzee in Sierra Leone is morphologically indistinguishable from *P. falciparum*, there is as yet no evidence that it is this species.

Mesnil (1920) failed to infect a chimpanzee by intravenous injections of human blood infected with *P. falciparum*. He also failed to infect the same chimpanzee by the bite of Anophelines with sporozoites of *Plasmodium falciparum* in their salivary glands.

Blacklock and Adler (1922) failed to infect:—

(1) Two Europeans by intravenous and subcutaneous injections of heavily infected blood from a chimpanzee.

(2) A chimpanzee by an injection of 3 c.c. of blood heavily infected with *P. falciparum* from a patient during his first attack of malaria, which he acquired in Sierra Leone.

(3) *Anopheles costalis* by feeding on a chimpanzee; but it should be noted that crescents were scanty in the animal's blood.

The existence of a relationship between human malaria and malaria in chimpanzees cannot be conclusively proved or disproved, until the insect vector of the latter be discovered and experiments with the infective vector carried out on human beings.

My best thanks are due to Mr. W. Addison, Provincial Commissioner of Kennema, and Mr. N. C. Hollins, District Commissioner of Pendembu, through whose kindness I obtained a number of chimpanzees.

SUMMARY AND CONCLUSIONS

Thirteen chimpanzees were examined for malaria in Sierra Leone.

Two young animals were found to be infected with a parasite indistinguishable from *P. falciparum*.

Older animals were negative, and resistance following attacks in early life is, therefore, suggested.

Blood from one chimpanzee containing only crescents failed to infect another chimpanzee.

Both infected animals on post-mortem examination showed fatty changes in the liver.

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EXPLANATION OF PLATE II

Malaria Parasites.

Figs. 1 to 17. Ring forms.

Figs. 18 to 19. Crescents.

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EXPLANATION OF PLATE III

Fig. 1. Micro-photograph of liver (Case II), showing fatty degeneration and infiltration. × 250.

Fig. 2. On the right, young chimpanzee with malaria, showing emaciation. (Note absence of paunch.)
On the left, healthy young animal.

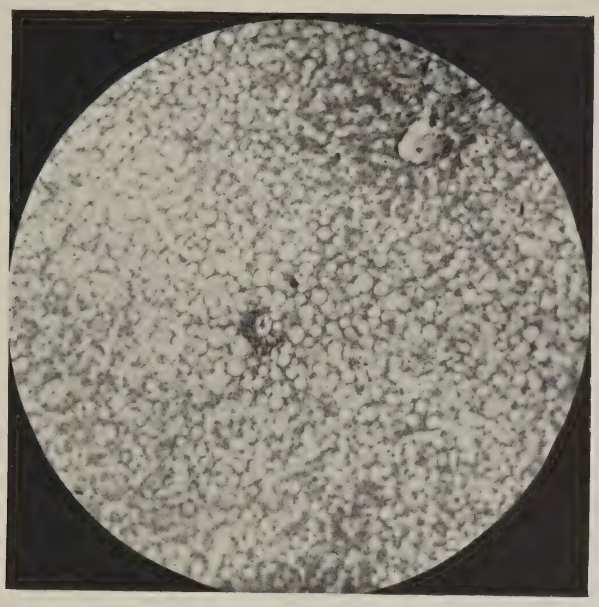

FIG. 1

FIG. 2

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NEW AND OLD OBSERVATIONS ON CERATOPOGONINE MIDGES ATTACKING OTHER INSECTS

BY

F. W. EDWARDS

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During the last two decades a fairly extensive literature has been published in regard to the attacks made by Ceratopogonine midges upon other insects. Several cases were placed on record by Knab (1914), who also reviewed the literature published previously to this date. More recently the facts have been reviewed by Peyerimhoff (1917) and Kieffer (1922), the former author adding some very interesting observations of his own. There are, however, one or two additional and very interesting cases which have been overlooked by all the above-named writers, and also some further unpublished instances which have come to my notice. It may, therefore, be worth while, in recording the fresh cases, to review again the literature of the subject.

The attacks made by midges upon other insects fall under two main heads:—

(1) *Cases of predacity*, where the midges attack other adult insects of approximately their own size, or not much larger, and kill them by puncturing their skin and sucking them dry. A good many instances of this have been noted, and I have summarised them in a recent paper (1920).

The following list gives the names of these species and of their victims :—

Predator	Victim
	NEUROPTERA
Palpomyia flavipes, (Mg.)	Ephemerid (*Baetis* sp.)
,, sp.	Perlid
	CHIRONOMIDAE
Bezzia annulipes (Mg.)	*Tanytarsus sylvaticus*, v.d. Wulp.
Probezzia multiannulata (Strobl.)	*Culicoides circumscriptus*, Kieffer.
Probezzia ? *signata* (Mg.)	*Culicoides pulicaris* (L.)
Stilobezzia gracilis (Hal.)	*Cricotopus pulchripes*, Verr.
,, ,,	*Orthocladius* sp.
,, ,,	*Tanytarsus*, 2 spp.
,, ,,	*Tanypus binotatus*, Mg.
Serromyia femorata (F.)	*Cricotopus pulchripes*, Verr.
,, ,,	*Bezzia ornata* (Mg.)
,, ,,	*Serromyia femorata* (F.) ♂
* ,, ,,	*Trichocladius* sp.
†*Ceratopogon candidatus*, Winn.	*Trichocladius* sp.
Ceratopogon lacteipennis, Zett.	*Camptocladius* ? *gracilis*, Goet.
,, ,,	*Culicoides arcuatus* (Winn.)
,, ,,	*Ceratopogon lacteipennis* Zett., ♂

In addition, Kieffer (1922) quotes Loew to the effect that *Macropeza albitarsis*, Mg., preys upon other small insects.

This list could no doubt be greatly extended by careful observation, and it seems probable that all the members of the bare-winged genera of *Ceratopogoninae* are normally predaceous in the female sex. Evidently the various modifications of the legs, such as swollen and often spiny femora, enlarged claws and spines on the last tarsal segments, which the females of most of these genera exhibit, are to be regarded as adaptations for holding their insect prey. It is probable that these predaceous habits are primitive in this sub-family, and that they have directly or indirectly led to the more specialised blood-sucking habits of certain species and genera.

Although it is beyond the scope of this paper, attention may be called in passing to the observations of Ingram, who found in West

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* Noted in North Cornwall, June, 1922. This is the only fresh record I have to add to the list previously published.

† Goetghebuer's review (1922) of the *Ceratopogoninae* in Meigen's collection has made it clear that *C. communis*, Mg., the type of the genus, belongs to Kieffer's genus *Psilobelea ;* this name, therefore, falls as a synonym of *Ceratopogon.* As I have stated in a recent paper (1921) I do not consider the differences between *Ceratopogon* (*Psilobelea*) and *Isobelea* are of more than subgeneric value, hence I include *I. lacteipennis* and its allies also in *Ceratopogon.*

Africa the larvae of *Forcipomyia ingrami*, Carter (1919), attacking mosquito larvae. This is, I believe, the only known instance of predacity in a *Ceratopogonine* larva.

(2) *Cases of blood-sucking*, where the attacking midge sucks the juices of its victim, without as a rule killing it, the victim in such cases being generally much larger than the attacking species. It is this class of phenomena with which I wish to deal more particularly in the present paper. Following the example of Peyerimhoff (1917), we may consider these midges in several groups, according to the type of host which they attack.

I. SPECIES ATTACKING MOSQUITOES

A considerable number of observations have been made on the relations between adult mosquitoes (generally *Anopheles*) and a species of *Culicoides* which is widely spread in the Oriental region. In a recent paper (1922) I have summarised these observations, and have described the midge concerned as *Culicoides anophelis*. It appears that the object of the Culicoides is to obtain engorged blood from the abdomen of its host, though it has in some cases been found to have attacked mosquitoes which were not engorged. At present only this single species of *Culicoides* is known to have these very remarkable habits.

This extremely interesting case may be regarded in one of two ways. It may be a development directly from a primitive predacity; the species having passed from a diet of (say) *Chironomidae* to one of mosquitoes, and thence to the mammalian blood contained in the body of its host. In this case it is easy to imagine that the midge might follow its mosquito host to its feeding ground, and eventually take to sucking blood itself directly from the mammal, thus giving rise to the blood-sucking habits now so general in the genus *Culicoides*. The possibility of this having been the course of development is somewhat strengthened by the fact that *C. anophelis* appears to show some somewhat primitive characters, such as the simple wing-pattern and rather large radial cells. On the other hand, it may be that the habit of obtaining blood from mosquitoes is purely secondary, and derives from an ordinary direct method of blood-sucking; this is, perhaps, most probable, since Lamborn's observations seemed to show that a blood meal was essential to the production of a complete fertile batch of eggs.

II. SPECIES ATTACKING ADULT *LEPIDOPTERA*

One instance has been recorded (by Kryger, 1914, quoted also by Knab, 1914) of a midge attacking a moth. The host was *Cidaria didymata*, L.; the midge was not precisely identified, but was stated by Knab to be apparently an undescribed species, 'belonging in the neighbourhood of *Ceratopogon murinus*, Winn.' In the hope of obtaining some further information concerning this species, I wrote to Mr. Kryger in Denmark, and also to Messrs. Aldrich and Böving in Washington, but only to discover that the material had been lost.

A second very similar case was discovered by Professor Newstead in North Wales in 1914, and I am greatly indebted to him for kindly allowing me to examine and describe the material of this most interesting find. While collecting at night with the aid of an acetylene lamp, Professor Newstead came across a cabbage-white butterfly whose wings were being attacked by nine specimens of a Ceratopogonine midge. The butterfly was considerably damaged, and as is shown by the accompanying photograph (fig. 1) the

FIG. 1. *Pieris napi* (slightly enlarged), victim of *Forcipomyia* (*Euforcipomyia*) *papilionivora*, Edwards. The left forewing, just below the costa and along both sides of the large vein, shows the nature of the damage, caused by the midges

damage would seem to have been caused, at least in part, by the attacks of the midges.* The latter appeared to be eating the wings

* Blood was seen exuding from the ruptured veins when the insect was captured; and the scales on either side of the veins are stained russet-brown, due apparently to the exudation. When first imprisoned the midges left their victim and swarmed over the glass lid of the collecting box; but on placing them in the dark, they were found, two hours later, to have resumed their attacks on the butterfly. (R. Newstead).

of the butterfly, though, as in the case of the Danish insects, they may in reality have been sucking juices from the wing-veins, especially if the blood was exuding from the broken ends of the veins.

After a careful examination of the literature, I have come to the conclusion that the midges collected by Professor Newstead belong to an undescribed species, and I, therefore, name and describe it as follows :—

Forcipomyia (*Euforcipomyia*) *papilionivora*, sp. n.

Head rather densely clothed with golden pubescence. Eyes practically touching, perfectly bare. *Antennae* uniformly dark, flagellum clothed with longish dark hair, nearly twice as long as the diameter of the segments. First eight flagellar segments together much shorter than the last five together (proportions 3 : 5). First flagellar segment nearly globular, the next five slightly transverse, seven and eight again practically globular, nine to thirteen each nearly three times as long as broad, thirteen with a nearly globular, nipple-like tip; one to eight each with rather long and stout sense-bristles, difficult to observe. *Palpi* dark, the second segment oval, broadest in the middle, not quite twice as long as its greatest breadth, apical part not suddenly narrowed; last two segments together as long as the second, the fourth a little longer than the third. Second segment with a globular internal cavity opening by a small round pore on the inner face. *Mandibles* broad, about three to five times as long as their greatest breadth, tip rather bluntly rounded, with about twelve to fifteen small, equal-sized teeth on one side; in the middle is an oval clear spot enclosing an elongate dark mark, resembling that figured by Carter, Ingram and Macfie in the genus *Prionognathus*. *Maxillae* almost as long as the mandibles, with about twenty-five fine regular crenulations, scarcely teeth, on one margin. *Hypopharynx* rather elongate, oval, a little over twice as long as broad, tip smooth. *Thorax* with the integument dull blackish, the humeral angles and the whole scutellum dull yellow. *Mesonotum* densely covered with short, bright golden pubescence mixed with longer, but not very long, brownish hair. Scutellum similarly but less densely clothed, postnotum shining black. *Abdomen* rather narrow for the genus, dull dark brown, uniformly clothed with short blackish hair. *Spermathecae* large,

nearly globular, necks practically without chitinisation. Cerci dark. *Legs* slender, practically uniform in colour, rather dark brownish, very hairy, the tibiae with some long hairs which are about six times as long as the tibial diameter. On all the legs the first tarsal segment is 2·5 times as long as the second. Empodia well developed, almost as long as the claws. *Wings* clothed rather densely (but somewhat less densely than in most species of the genus) with close-lying dark hair; most of the hair on the thick veins golden, but mixed with some dark. Venation normal for the genus: *Rs* in contact with *R1*, so that the first radial cell is obliterated; second radial cell about twice as long as broad, trapezoidal; petiole of median fork a little shorter than the very oblique r-m; cubitus forking below end of costa, which reaches just beyond the middle of the wing. *Halteres* with the stem dark, the knob white.

Length of body, 1·8 mm.; wing, 1·4 mm.

NORTH WALES: Ty Gwyn Farm, Aberhosan, Machynlleth, found at 10.15 p.m. feeding on the wings of *Pieris napi* (R. Newstead). Three ♀ co-types in the British Museum, presented by the collector; six others in the collection of the Liverpool School of Tropical Medicine.

This insect seems to have no very close ally among the European species. By Kieffer's table it will run down to *F. formicaria* (Kieff.), which differs in having the first tarsal segments much shorter, as well as in the palpal structure and other details. Other European species which show some points of resemblance are *F. hirta* (Lundst.) and *F. murina* (Winn.), but none show the combination of antennal and tarsal characters possessed by this species. In both these respects the new species resembles *Lasiohelea velox* (Winn.), but it has not the venation of the genus *Lasiohelea*; it belongs to Malloch's group *Euforcipomyia*, and bears a close resemblance to the North American *E. fusicornis* (Coquillett) (see below).

From the above remarks it will be seen that the specimens collected by Professor Newstead might have been referred to as 'an undescribed species belonging in the neighbourhood of *Ceratopogon murinus*, Winn.,' and it, therefore, seems not improbable that the Danish specimens found by Mr. Kryger may have belonged to the same species. In any case, it is interesting

to note that the only two records we have of midges attacking adult *Lepidoptera* both refer to an insect belonging to the same group of the genus *Forcipomyia*.

III. A SPECIES ATTACKING A SIALID

Malloch (1915) states that he has seen a specimen of *Euforcipomyia fusicornis* (Coquillett) which was taken attacking a Sialid (*Chauliodes* sp.). In view of the large size of the victim, this must be classed as a case of blood-sucking rather than of predacity. It is not stated what part of the Sialid was attacked, but it is evident that we are here dealing with a very similar case to the two last considered, *Chauliodes* being a large-winged, rather soft-bodied insect, comparable with a moth. It is, therefore, of special interest to note that *E. fusicornis*, according to Malloch's description, bears a very close resemblance to the species just described as *F. papilionivora*. In fact, it is not impossible that the two may be conspecific, though it seems unsafe to identify a European with a North American form without actual comparison of material.

Although I do not consider *Euforcipomyia* to be generically distinct, it may be retained as a sub-genus in the sense in which Malloch proposed it: i.e., to include the species of *Forcipomyia* which have the first hind tarsal segment markedly longer than the second, reserving *Forcipomyia* (*s. str.*) for those species in which the first is shorter, or at most slightly longer, than the second. This is not the sense in which Kieffer has used the name, but seems to be the correct one, since the type species of *Forcipomyia* is *bipunctata*, L. (*trichoptera*, Mg.), not *albipennis*, Mg., as stated by Kieffer.

IV. SPECIES ATTACKING CATERPILLARS

A number of cases of midges attacking caterpillars have been recorded from time to time. Most of these were referred to by Knab (1914), the cases he mentioned being as follows:—

Species	Host	Observer
Forcipomyia propinqua (Will.)	*Melanchroia geometroides* (Waker) (*Geometridae*)	Baker (Cuba)
F. squamosa, Lutz.*	Sphingid (undetermined)	Townsend (Peru)
F. sp.	Sphingid (undetermined)	Barbiellini (Brazil)
F. crudelis, Knab.†	Not stated	Urich (Mexico)
F. erucicida, Knab.	Erinnyis ello L. (*Sphingidae*)	Mosier (Florida)

* Specific name given by Lutz (1914).

† This specific name is preoccupied by *F. crudelis* (Karsch), but I refrain from proposing a substitute because the descriptions appear to indicate that the species is almost certainly identical with *F. tropica*, described by Kieffer (1917) from Costa Rica.

All the above-named species of *Forcipomyia* belong to that group of the genus in which the female tibiae are devoid of scales, and the second segment of the hind tarsi is at least twice as long as the first. The same remark is true of three other species which were not known to Knab, and are discussed below. It would seem, therefore, that the habit of attacking caterpillars is a very special one, restricted to this group of the genus *Forcipomyia*.

Forcipomyia crudelis (Karsch, 1886)

This species was described from a single female found by Karsch sucking a saw-fly larva in the neighbourhood of Berlin. He remarks that his attention was called to the larva by the movements which it made in endeavouring to dislodge its tormentor, and that the latter had its mouth-parts so firmly fixed in the body of its victim that it did not loose its hold even when the pair were placed in the cyanide bottle. *F. crudelis* has not been recognised since Karsch described it, but it is evidently very closely related to *F. pallida* (Winn.), *F. brevimanus* (Lundst.) and *F. alboclavata* (Kieffer).

Forcipomyia hirtipes (de Meij.)

Two females of this species were found by Mr. J. C. F. Fryer (recorded by me, 1913), at Peradeniya, Ceylon, each sucking a larva of *Papilio clytia*. *F. hirtipes*, it is interesting to note, closely resembles the European *F. alboclavata*, showing only very slight differences in the proportions of the palpal and tarsal segments. The Ceylon specimens do not agree with de Meijere's description, as regards the middle tarsi; but, as he has informed me, the description is incorrect. In reality, he says 'the mid-tarsal segments have about the same proportions as the hind, viz., in ♂ about 8 : 30 : 14 : 11 : 8, in ♀ about 9 : 25 : 11 : 9 : 8.' He also informs me that though the antennae of the type ♀ are mutilated, in another specimen the proportion of the first eight to the last five flagellar segments is about 27 : 38. These proportions are about the same as in the Ceylon specimens.

Forcipomyia alboclavata (Kieffer, 1919), (*canaliculata*, Goetghebuer, 1920)

The British Museum possesses three ♀♀ of this species from Taterafuered, Hungary, 1906 (*Hon. N. C. Rothschild*), on which

the donor sent the following note:—'Sitting on the backs of larvae of *Deilephila galii* (which were extremely common in a large field near Taterafuered), and appearing to eat some secretion from their skins.' The specimens have the second hind tarsal segment 2·5 instead of only twice as long as the first, but otherwise agree with Keiffer's description, and I have no hesitation in quoting the synonymy as above.

F. alboclavata has been found in Scotland (Arran) as well as Belgium, but its habits in these countries have not been observed.

V. SPECIES ATTACKING OIL-BEETLES

Peyerimhoff (1917) has given an interesting account of the relations between a Ceratopogonine midge (at present undetermined) and the oil-beetle *Meloe majalis*, L., in Algeria. The flies, he says, pursue these large beetles in little swarms, and without inconveniencing them in any way, feed upon their yellow blood. M. de Peyerimhoff informs me that the flies are now in the hands of Professor J. J. Kieffer, who believes that they represent a new species.*

More recently, a second similar instance, this time from Denmark, has been recorded by Hansen (1921). I am indebted to my friend, Mr. J. P. Kryger, for the following translation of Mr. Hansen's note:—

'A gnat attacking a *Meloe*—29th May, 1921. I saw a *Meloe proscarabaeus* crawling along a walk in the wood of Ulvlyst (Denmark). A little swarm of gnats hovered over the beetle and sometimes attacked it, especially on the soft skin between the first and second thoracic segments. The beetle was seriously affected by the gnats biting, and rubbed its sides with its hind legs, but without getting rid of its tormentors. When I put the collecting bottle over the beetle two gnats were sitting on its back, but as it tumbled in twelve gnats appeared in the bottle. The remaining ten must have been sitting on the underside of the beetle.'

Mr. Kryger has further been so good as to obtain for me the loan of the specimens captured by Mr. Hansen, which had been

* Since this was written I have received, through the kindness of Professor M. Bezzi, a number of the specimens originally collected by M. de Peyerimhoff. Without trespassing on ground to be covered by Professor Kieffer, I may remark that these specimens represent a species which is extremely nearly related to *Atrichopogon rostratus* (Winn.), a fact which is of much interest in view of my determination of the Danish specimens.

presented by the collector to the Zoological Museum at Copenhagen. Upon examination of the flies I find that they belong to the species *Atrichopogon rostratus* (Winn.), all, of course, being females. The purpose of the formidable proboscis possessed by this species thus becomes apparent for the first time, for neither it nor any other member of its genus has been known either to bite warm-blooded animals or to prey upon other small insects. But, as in the case of many other midges with strong food preferences, the diet of *A. rostratus* is not confined to the blood of *Meloe*, but consists partly of vegetable substance (honey, or perhaps pollen). All the adult specimens of this midge which I have found myself have been taken on the flowers of umbellifers (*Angelica* and *Heracleum*), often in company with great numbers of some other species of *Atrichopogon*.

VI. A SPECIES ATTACKING A PHASMID

Williston (1908) mentions a minute fly which was found in the West Indies 'closely applied to and apparently sucking the juices from the antennae of a Phasmid.' He considered the specimen to represent a new genus of *Simuliidae*, but the figures which he gives indicate rather a Ceratopogonine midge. The available evidence is insufficient to place this species generically, though if Williston's figure of the wing is accurate, it would not seem to fit very well into any known genus. The specimen is not among the West Indian collections in the British Museum which were studied by Williston, and I am informed that it cannot be traced in those parts of his collection which are now in Washington and New York.

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ON SOME STRONGYLID LARVAE IN THE HORSE, ESPECIALLY THOSE OF *CYLICOSTOMUM*

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The adult stage of species of *Cylicostomum* found in the large intestine of the horse has been extensively studied by Looss and, more recently, by Boulenger, and Yorke and Macfie (cf. Ihle, 1922), but very little is known as yet of the development of these species in the body of the horse.

The larvae of *Cylicostomum* are to be found in large numbers in the mucosa of the caecum and colon of the horse. They were first found by Dick (1836) and described by Knox (1836). By Diesing (1851, Vol. II, p. 332) they were mentioned under doubtful species as *Nematoideum equi caballi*. T. Spencer Cobbold (1874, p. 85) describes and figures *Cylicostomum* larvae as adult Nematodes under the name *Trichonema arcuata*, but the next year (1875, p. 241) he states that *Trichonema* is only the larval form of *Cylicostomum* (' *Strongylus tetracanthus* ').

Short descriptions and sketches of the larvae, encysted in the mucosa of the large intestine, are also to be found in Leuckart (1876, p. 445), Cobbold (1886, p. 288) and Giles (1892, p. 15, Pl. III, figs. 16, 18). The last-mentioned author thought he had found a free-living *Rhabditis* generation of *Cylicostomum*. This mistake, which has been made repeatedly and also recently, when the development of different Nematodes was traced, is due to the fact that the cultures of larvae were infected with free-living Nematodes.

An investigation of Cuillé, Marotel and Roquet (1913), dealing with our subject, is of more importance than the older publications above mentioned. These authors distinguished three types of larvae, living in the mucosa of the large intestine of the horse and considered as belonging to *Cylicostomum*:—(1) 'La larve oesophagostomiforme,' with mouth-capsule and dorsal tooth and having a length of 2 to 5 mm.; (2) 'la larve metastrongyliforme,' without mouth-capsule (length 800μ to 2 mm.); and (3) 'embryons,' without recognisable internal structure (length 300μ to 800μ). They showed that the 'larve oesophagostomiforme' passes over into the juvenile *Cylicostomum* by a moult.

Recently a part of the development of *Cylicostomum insigne* was shortly described by Boulenger (1921), who figures small larvae (6 to 7 mm. in length) and large larvae (up to 11 mm. in length), both agreeing with the 'larve oesophagostomiforme' of the French authors. In the larger larvae the adult mouth-capsule makes its appearance, which represents the preparation for the last ecdysis.

We ourselves have examined a large number of larvae, partly collected by the Commission appointed to inquire into Sclerostomiasis in Holland, and partly by ourselves in the horses dissected in the Anatomical Institute of the Veterinary College at Utrecht. All the larvae were found in the mucosa of the large intestine, though a small number were met with free in the lumen of the intestine.

The larvae examined by us can be divided into different types, to be described in subsequent pages. They belong for the greater part to *Cylicostomum*, a few perhaps to *Triodontophorus*; others were not identified. In addition we found a few very small larvae without recognisable internal structure. They agree with the so-called 'embryons' of Cuillé, Marotel and Roquet (1913, p. 8 of reprint), and were only obtained by us in a few cases by scratching the mucosa of the large intestine. We have not yet studied these forms in detail, but we do not think that these small worms (according to the French authors measuring 300μ to 800μ in length) must be considered to belong to the genus *Cylicostomum*, because it follows from the investigations of A. Albrecht (1909) and of De Blieck and Baudet (not yet published) that the larvae of *Cylicostomum* infecting the horse are much more differentiated.

CYLICOSTOMUM LARVAE

All *Cylicostomum* larvae, found by us in the mucosa or in the lumen of the intestine, show a cup-shaped larval mouth-capsule, which has been already described by Cobbold (1874, p. 86). In agreement with this author (1886, p. 288) we will call this larval stage *Trichonema* stage. The name 'larve oesophagostomiforme' must be rejected, as these larvae do not in any particular agree with *Oesophagostomum*.

The cuticle is ringed. The cuticle surrounding the circular mouth-opening may also be called mouth-collar here; this larval mouth-collar, however, is much less developed than the adult one. The mouth-opening is generally surrounded by six papillae. External and internal leaf-crown are absent. The mouth-capsule is either sharply marked off from the mouth-collar or passes gradually over into it. In the middle the mouth-capsule is mostly wider, and possesses a thicker wall than posteriorly and anteriorly. Especially near the mouth-opening, the wall is very thin. The anterior part of the mouth-capsule is mostly provided exteriorly with a collar, often strongly developed, and which we will call mouth-capsule collar. It is divided into six lobes, which have a crescent shape and are almost perpendicular to the outer surface of the mouth-capsule. Between every two lobes a head-papilla is to be found.

An oesophageal funnel, in which the three sectors of the oesophagus continue, is present. The dorsal sector is always provided with a tooth, more or less protruding into the lumen of the mouth-capsule. The cuticular lining of the anterior margin of the oesophageal funnel shows a circular thickening, adjacent to the mouth-capsule. We will call this thickening the funnel-ring; it is directed to the exterior.

The oesophagus is cylindrical in shape and somewhat swollen posteriorly. Where the oesophagus passes over into the mesenteron three valves protrude into the lumen of the intestine. A nerve-ring, surrounding about the middle of the oesophagus, is present.

The mesenteron is composed for the greater part of a dorsal and a ventral row of alternating, polynuclear cells, which are mostly pigmented. In the anterior part of the mesenteron the cells are always much flatter than in the posterior part. In the anterior part

the cell-limits run transversely or directed obliquely to the front; so that the lateral parts of the cell-limits are situated more anteriorly than the dorsal and ventral parts.

The very short rectum opens into the exterior through the anus, situated at a small distance from the sharp posterior extremity of the body.

Sometimes, but not always, the larvae living in the mucosa are red in colour. It appears that in the few cases examined by us the whole body, the pigmented intestine excepted, is red. When such a larva is pricked, a red fluid is emitted. The juvenile specimens, living in the lumen of the colon and caecum, may also show this colour, but, as in the case of the larva, the intestine was not red in the specimens examined by us. Prof. B. Sjollema and Miss J. E. van der Zande were so kind as to analyse microchemically and spectroscopically a few juvenile specimens of *Cylicostomum insigne* for us. The fluid appeared to be due to oxyhaemoglobin.

We assume that the larvae living in the mucosa feed on blood at least during a part of their life. Boulenger (1921, p. 324) found these larvae in cysts, filled with blood; this is not always the case, however. As mentioned above, the red colour was not observed in the cells of the intestine of the larvae. We suppose that the larvae had fed on blood in an earlier period; consequently the red colour must have disappeared already from the intestinal wall, but not yet from the rest of the body. Further, we are of opinion that the red colour of the adult worm is the consequence of the larvae having fed on blood, for the adult *Cylicostomum* feeds on the contents of the large intestine of the host and not on blood. After dissection these worms are never found attached to the mucosa of the host's intestine.

The larvae, which we consider to belong to the genus *Cylicostomum*, can be divided into two types, to be described below. Not much importance must be attached to the dimensions indicated, as larvae of numerous species are brought together which when adult differ strongly in size. We cannot state to which species of *Cylicostomum* these different types belong, because we have not at our disposal a large enough number of moulting specimens.

Cylicostomum Larva. Type A (fig. 1).

To this type the smallest larvae of the genus *Cylicostomum* are considered to belong, having a length of 3 to 4·5 mm. and a maximum thickness of 110μ to 200μ. The mouth-margin is smooth. Around the mouth-opening the cuticle is thick. Head-papillae could not be observed. A mouth-capsule collar was not found by us. The length of the mouth-capsule, including the mouth-collar, varies from 20μ to 28μ.

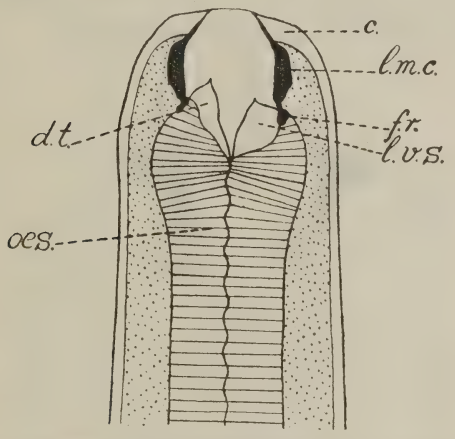

Fig. 1. Anterior extremity of a *Cylicostomum*-larva, type A, seen from right side. × 540 (× $\frac{3}{4}$). *d.t.*—Dorsal tooth; *oes.*—Oesophagus; *c.*—Cuticle; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—funnel-rings; *l.v.s.*—Latero-ventral sector of the oesophageal funnel.

In this type the oesophageal funnel also possesses three sectors. The dorsal sector always possesses a tooth, varying in size; the two latero-ventral sectors are rounded or bear an inconspicuous tooth, which never protrudes as far into the lumen of the oral capsule as the dorsal, large tooth. The length of the oesophagus varies from 250μ to 350μ. The distance from the anus to the posterior extremity of the body is 80μ to 130μ.

This type is very common.

Cylicostomum Larva. Type B (fig. 2).

Another type (B) is also of frequent occurrence. It differs from Type A in being of a larger size and in possessing a mouth-capsule collar. The length is 7·5 to 12·5 mm., the maximum thickness

420μ to 580μ. Six head-papillae are present, agreeing as to arrangement with those of the adult specimens; so there are two lateral and four sub-median papillae. The oral margin is mostly somewhat incised near the six papillae. Length of the mouth-capsule, including the mouth-collar 55μ to 65μ. At one-third of the length of the mouth-capsule from the posterior margin the wall of the oral capsule is thickest. The wall of the mouth-capsule becomes thinner anteriorly and passes gradually over into the mouth-collar. The mouth-capsule collar is very well developed and situated immediately under the cuticle of the anterior part of the body. Here the cuticle is somewhat thickened.

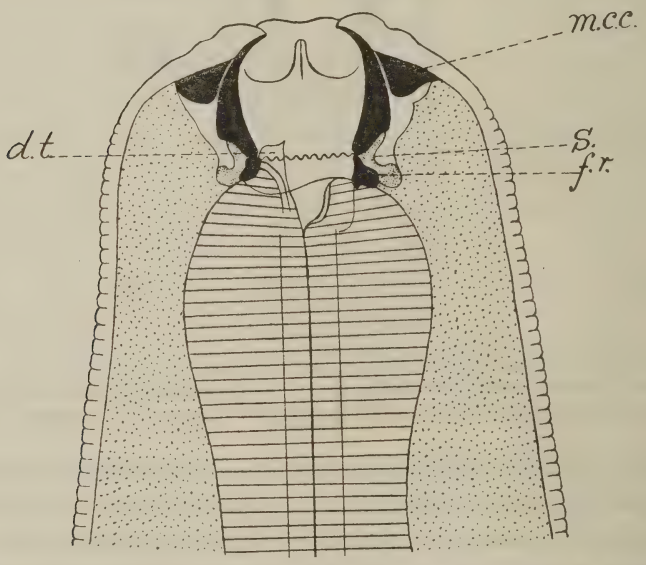

FIG. 2. *Cylicostomum*-larva, type B, seen from right side. × 435 (× $\frac{2}{3}$). *d.t.*—Dorsal tooth; *m.c.c.*—Mouth-capsule collar; *s.*—Septum; *f.r.*—Funnel-ring.

The oesophageal funnel is conspicuous and bears a cuticular lining of variable thickness. The funnel-ring is well developed. In some cases the border between mouth-capsule and funnel-ring is irregular or undulating. Sometimes the funnel-ring possesses a circular groove at its outer surface, so that in optical section it appears as a double ring. In this type, too, the dorsal sector of the oesophagus is continued as a tooth, protruding into the lumen of the oral capsule; this tooth is relatively not so large as in Type A.

The latero-ventral sectors are truncated anteriorly, or become lower and lower, to end at the funnel-ring. At the bottom of the grooves by which the sectors are separated the cuticle is thickened, just as in the adult worm. The oesophagus measures 550μ to 650μ in length; the distance from the anus to the extremity of the body is 190μ to 220μ.

We consider that the larvae belonging to Type B represent a more developed stage of Type A, because we have found several larvae with the rudiments of the mouth-capsule collar (fig. 3); these

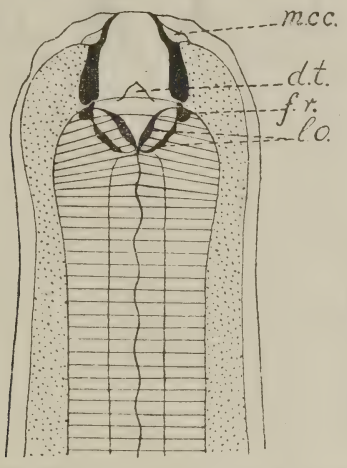

Fig. 3. *Cylicostomum*-larva, intermediate between types A and B, seen from dorsal side. × 540 (× ¾). *m.c.c.*—Mouth-capsule collar; *d.t.*—Dorsal tooth; *f.r.*—Funnel-ring; *l.o.*—Lining of the oesophageal funnel.

larvae are of a size intermediate between those of Types A and B. Length 5 mm. to 6·5 mm., maximum thickness 350μ; length of the mouth-capsule, including the mouth-collar, 42μ; oesophagus 540μ long.

The last ecdysis (figs. 4, 5).

The *Trichonema* stage passes over into the juvenile worm, living in the lumen of the large intestine, by a moult. In agreement with the development of other Nematodes, we assume that this moult is the fourth and last. The ecdysis itself takes place in the intestinal lumen.

The moult begins with the formation of a cavity around the larval mouth-capsule (fig. 2). We consider that one continuous cavity is

present from the beginning. Boulenger (1921, p. 325) mentions a series of cavities. However, according to Looss (1897, p. 925), two cavities (a dorsal and a ventral one) are formed in the larva of the fourth stage of *Ancylostoma*. Later on these cavities unite to form a circular lumen.

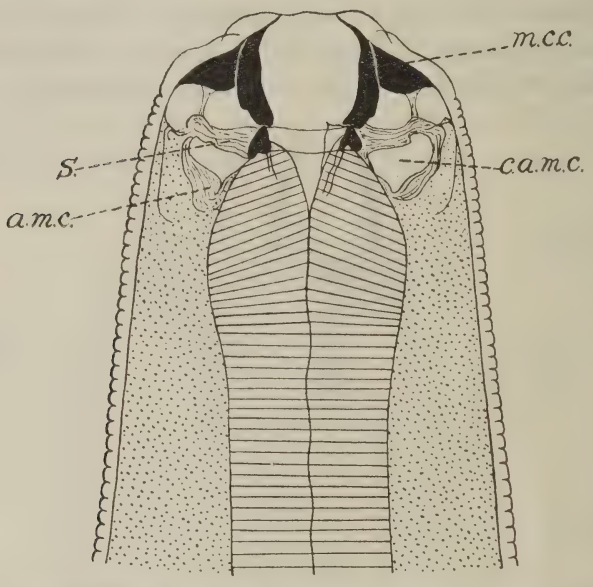

Fig. 4. *Cylicostomum*-larva, type B, seen from left side, with rudiment of the cavity of the adult mouth-capsule. × 290 (× ¾). *s.*—Septum; *a.m.c.*—Wall of the adult mouth-capsule; *m.c.c.*—Mouth-capsule collar; *c.a.m.c.*—Cavity of the adult mouth-capsule.

When the cavity makes its appearance, it is narrow at the front and a little wider backwards. Later on the anterior part of this cavity extends to the mouth-capsule collar. In the posterior part, which extends almost to the oesophagus, we see a granular substance, which seems to form a thin layer (fig. 2, *s.*) about at the level of the posterior margin of the mouth-capsule. This layer corresponds with the definitive anterior side of the mouth-capsule of the adult worm. This septum (fig. 4, *s.*) gradually becomes thicker, possibly formed by the granular substance mentioned above, while the cavity lying behind this septum, and in the beginning filled up with this substance, becomes empty and extends simultaneously backwards. This cavity, situated behind the septum, is the lumen of the adult mouth-capsule. At the periphery of the septum the definitive

mouth-collar and the definitive head-papillae develop (fig. 5). The cavity mentioned gradually widens and peripherally begins to form the wall of the adult oral capsule. Now this circular cavity surrounds the anterior part of the oesophagus (fig. 4).

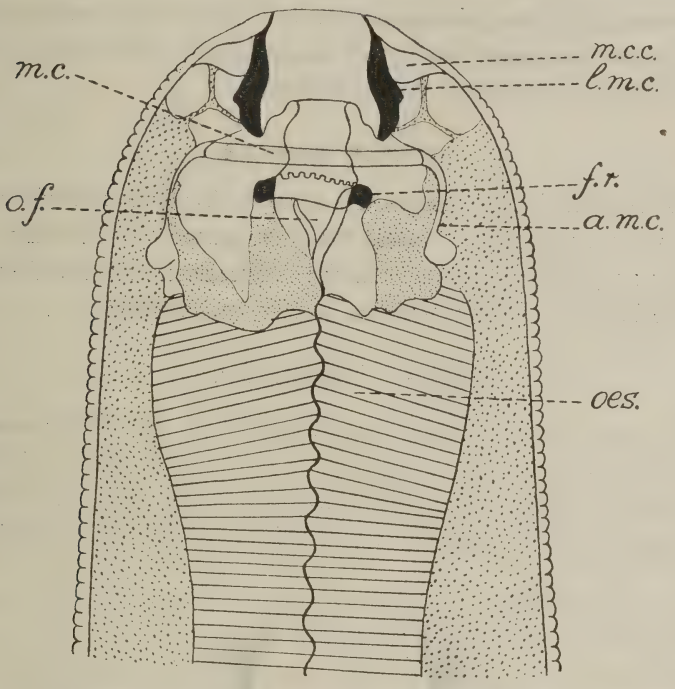

Fig. 5. *Cylicostomum*-larva, type B, moulting. × 290 (× ¾). *m.c.*—Mouth-collar; *o.f.*—Larval oesophageal funnel; *m.c.c.*—Mouth-capsule collar; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—funnel-ring; *a.m.c.*—Wall of the adult mouth-capsule; *oes.*—oesophagus.

Meanwhile the cuticle of the adult worm is formed below the provisional one. Before the ecdysis proper the oesophagus loosens itself from the cuticular lining of its funnel (fig. 5). In earlier stages the oesophagus tapers to the anterior extremity and ends at the funnel-ring (fig. 4). But at this stage it becomes truncated in front. Now the lumen of the mouth-capsule is situated before the oesophagus, whereas the anterior part of the latter was formerly surrounded by the adult mouth-capsule. Simultaneously the mouth-capsule and the funnel-ring, which remains connected with the cuticular lining of the provisional oesophageal funnel, separate. The posterior margin of the mouth-capsule and the anterior margin

of the funnel-ring remain connected by a thin membrane, of which the origin is difficult to trace. Boulenger (1921, fig. 5 *b*) also figures it, without describing it. In the moulting specimen sketched by Cuillé, Marotel and Roquet (1913, fig. 17, 7), in which the adult mouth-capsule and mouth-collar have developed completely, the funnel-ring and the provisional mouth-capsule are still connected. In the specimen sketched by us the external leaf-crown is already visible, but not indicated in the figure.

OTHER LARVAE

Besides the larvae Types A and B (*Cylicostomum*), we also found some other types, which we were unable to identify. Short descriptions, dealing only with the differences between these types and the larvae of *Cylicostomum*, will now be given.

Larva. Type C (fig. 6).

This type and the following Type D do not differ essentially from the larvae of *Cylicostomum*. Type C was found only once in the mucosa of the large intestine; but three specimens could be investigated. The length is 4·5 to 6·6 mm., the maximum thickness

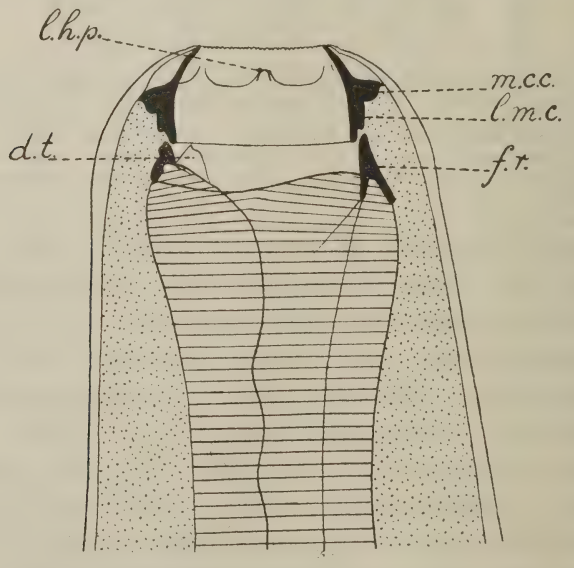

Fig. 6. Larva, type C, seen from right side. × 310 (× ¾). *l.h.p.*—Lateral head-papillae; *d.t.*—Dorsal tooth; *m.c.c.*—Mouth-capsule collar; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—Funnel-ring.

225μ to 380μ. The mouth-opening is circular, its margin is delicately denticulated. A mouth-collar is present, of which the side directed to the body-axis possesses a layer passing over into the anterior margin of the mouth-capsule. (In the figure the limit between mouth-collar and mouth-capsule is not indicated.) The head-papillae are present. The oral capsule is short and very wide; it is 52μ to 65μ in length, the mouth-collar included. The mouth-capsule collar is well developed and implanted in the anterior half of the mouth-capsule.

The oesophageal funnel is wide and bears a dorsal tooth. The latero-ventral sectors are smooth. The funnel-ring is very long at the dorsal side, shorter, however, than at the ventral side, where its length is equal to that of the mouth-capsule. In optical section this ventral part especially has the shape of an inverted Y, which encloses a part of the musculature of the oesophagus. The latter is short and thick, 435μ to 550μ in length, consequently measuring one-tenth to one-twelfth of the total body-length.

The posterior extremity is rounded. In one of the specimens examined, the part of the body situated behind the anus becomes gradually thinner; the distance from the anus to the posterior extremity is considerable (380μ) here. In two other specimens the thickness of this part diminishes suddenly at some distance behind the anus; consequently the body ends in an almost cylindrical point. In these cases the distance from the anus to the posterior extremity is 155μ to 180μ. Possibly these are sexual differences.

LARVA, Type D (fig. 7).

We found this type five times (only a few specimens) in the mucosa of the large intestine. We do not know whether this type and also the former (Type C) belong to *Cylicostomum*. Length 3·5 to 5·1 mm., maximum thickness 120μ to 190μ. The head-papillae are present. The cuticle is swollen around the mouth-opening; here the part of the cuticle directed to the body-axis possesses a particular layer, which passes over into the mouth-capsule. The latter is 25μ to 27μ long, the cuticle surrounding the mouth-opening included. Posteriorly its wall increases in thickness. A slightly developed mouth-capsule collar is present, lying immediately against the cuticle.

The dorsal sector of the oesophageal funnel bears a large tooth, protruding far into the lumen of the mouth-capsule. At the dorsal side its anterior margin possesses a small point and at the ventral side a large one. Each of the latero-ventral sectors bears a small tooth with one point. Moreover, the well-developed funnel-ring

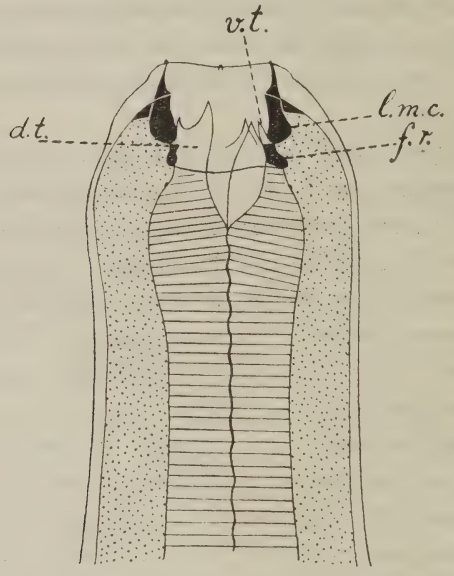

FIG. 7. Larva, type D, seen from right side. × 540 (× ¾). *d.t.*—Dorsal tooth; *v.t.*—Ventral tooth; *l.m.c.*—Wall of the larval mouth-capsule; *f.r.*—Funnel-ring.

possesses medio-ventrally a pointed tooth, being directed anteriorly (fig. 7, *v. t.*). Peripherally the funnel-ring does not protrude markedly. The oesophagus is long (400μ to 435μ), being one-ninth to one-twelfth part of the body-length. The mesenteron agrees with that of *Cylicostomum* larvae. The anus is situated 105μ to 115μ from the posterior extremity of the body.

LARVA. Type E (fig. 8).

We found this type only once in four specimens in the lumen of the large intestine. We consider that these larvae belong to *Triodontophorus*. It is, however, very remarkable that we found this type only once in our comprehensive material, as two *Triodontophorus* species are common in Holland, and sometimes inhabit one host in large quantities.

The length of these four larvae is 7·6 to 8·5 mm., the maximum thickness 310μ to 365μ. The mouth-opening is circular and surrounded by a thin mouth-collar, finely and longitudinally striated, and resembling an extremely little developed external leaf-crown. The six head-papillae are distinctly visible. The length of the mouth-capsule (including the mouth-collar) is 65μ to 82μ. The mouth-capsule is wide, cup- or barrel-shaped, and sharply marked

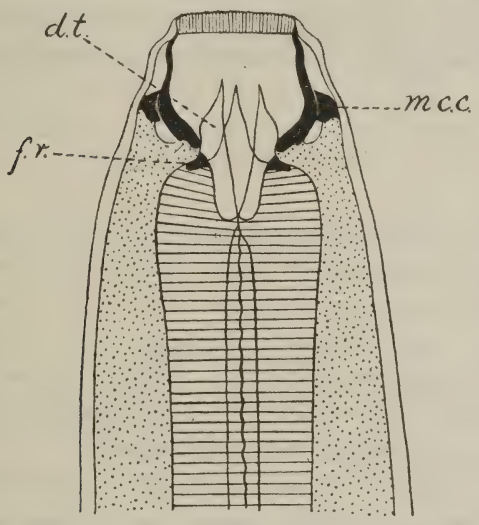

FIG. 8. Larva, type E, seen from right side. × 335. (× $\frac{3}{4}$). *d.t.*—Dorsal tooth; *f.r.*—Funnel-ring; *m.c.c.*—Mouth-capsule collar.

off from the mouth-collar. In some of the specimens it lies immediately against the cuticle. (The space between cuticle and mouth-capsule in the specimen figured is possibly due to the preservation.) The mouth-capsule collar reaches very far posteriorly beyond the equator of the mouth-capsule. Around the posterior part of the latter a circular cavity is already visible: the rudiments of the lumen of the adult mouth-capsule.

The oesophagus is long (750μ to 820μ), being about one-tenth of the body-length. The oesophageal funnel is well developed. It bears three large, pointed teeth, which protrude far into the lumen of the mouth-capsule. The dorsal tooth is a little larger than both the latero-ventral teeth. A funnel-ring is present. The distances from the anus to the rounded posterior extremity of the body is in

two specimens respectively 100μ and 110μ, in both other specimens respectively 220μ and 240μ; possibly these are sexual differences.

We suppose that these larvae belong to *Triodontophorus*, because the oesophageal funnel bears three large teeth protruding into the lumen of the mouth-capsule, this characteristic being present among the adult Strongylids of the horse, in *Triodontophorus* only. Moreover, the great length of the oesophagus of this type agrees with the long oesophagus in *T. intermedius* and *T. brevicauda.* For the rest, no other larva was present in our material which could be considered to belong to *Triodontophorus* on better grounds.

Larva. Type F (fig. 9).

Besides the larvae described above, which all possess a well-developed mouth-capsule, we found in the mucosa of the large intestine of the horse in one case one larva without mouth-capsule.

Length 3·7 mm., maximum thickness 190μ. In front of the oesophagus, having a length of 350μ, is a tube-shaped mouth-cavity, projecting slightly above the level of the anterior extremity of the body and being spherically swollen posteriorly.

We cannot decide whether this larva is identical with the 'larve metastrongyliforme' of Cuillé, Marotel and Roquet, which, however, is shorter (length 800μ to 2 mm.) than the specimen found by us.

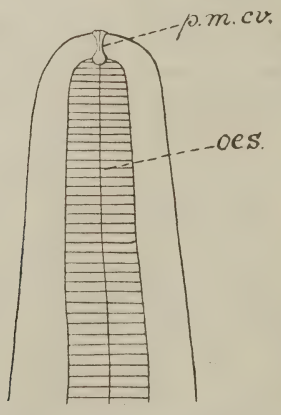

Fig. 9. Larva, type F. × 340 (× $\frac{3}{4}$). *p.m.cv.*—Provisional Mouth-cavity; *oes.*—Oesophagus.

Possibly both the larvae observed by the French authors and the specimen found by us are identical with a larva encountered by Leuckart (1876, p. 446) in the mucosa, being 1 mm. long and differing 'durch die Abwesenheit des Mundbechers, dessen Stelle durch einen schlanken und dünnhäutigen Chitincylinder vertreten war, wie bei den ersten parasitischen Jugendzuständen des *Dochmius trigonocephalus*. Die Umwandlung in die Form mit Mundbecher geschieht durch eine Häutung, die schon bei Exemplaren von 1·5 mm. vollendet ist.' If the supposition above made proves to be correct, Type F represents the third larval stage of *Cylicostomum*, though the differences between the encysted larvae (larvae of the third stage, enclosed in the cuticle of the second stage) and Type F are conspicuous.

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AVIAN CESTODES FROM NEW GUINEA

II. CESTODES FROM CASUARIFORMES

BY

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(*Received for publication* 5 *December*, 1922)

As it has been pointed out in the first part of this paper (Kotlán, 1921), the worms described in both the former and present notes, as well as those which will be described subsequently, belong to a rather large collection of parasites which were partly sent, partly brought back, by the Hungarian naturalist, Lewis Biró, from the formerly German New Guinea, during the years 1897-1899.

The intestinal parasites of birds belonging to the Casuariformes are represented in this collection by numerous Cestodes from *Casuarius picticollis*, Sclat. A hasty examination of these worms showed that they all belong to the family of Davaineidae. On account of external features two species could be distinguished, a larger and a smaller one, both belonging to the genus *Davainea*, R. Bl. (s. l.). Until now only one representative of this genus was known from Casuariformes,* viz., *D. australis* (Krabbe, 1869), from *Dromaeus novae hollandiae*. This species, however, is easily separated from the two above apparently undescribed forms.

DAVAINEA (s. l.) *CASUARII*, sp. n.

Host: *Casuarius picticollis*, Sclat.

Locality: Erima and Sattelberg.

The majority of the worms collected from this host—about two hundred more or less developed specimens—belong to this new

* Meggitt (1921) quotes in his key to the species of *Davainea two* ' *Davainea, sp. nov.*, Vevers, 1920 ' from *Casuarius uniappendiculatus* Blyth. I have been unable to obtain Vevers' paper (*Proc. Zool. Soc.*, 1920.).

species. The strobilae, coming from the two above-mentioned localities, exhibit in their external appearance a well marked difference, for those from Sattelberg are much more contracted and have a shorter, almost cylindrical body, while those from Erima are more stretched and thus longer in size. The largest specimens measure 34 cm., the greatest width (3 mm.) occurs in the posterior part of the strobila. The worms, which are in an expanded condition, bear a well marked scolex, which is short and approximately square, its diameter being 1 to 1·2 mm., while in contracted worms it is not clearly marked off from the strobila. It also happens sometimes that the anterior end of the strobila bears by means of unequal contraction a pseudoscolex-like thickening of 3 to 5 mm. length, with the true scolex at the end, as is shown in fig. 1. The

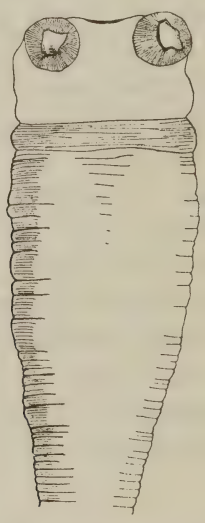

Fig. 1. *Davainea casuarii*, n.sp. Showing the extremely contracted anterior end of the body with the scolex. × 17.

rather muscular rostellum measures 0·5 mm. in breadth, and is armed with two hundred and fifty very large hammer-shaped hooks, which are arranged in two rows. The hooks of the anterior row are 48μ to 54μ, those of the posterior row 40μ to 46μ in length. As far as I am aware, there is only *Houttuynia struthionis* (Houtt.) which has larger hooks, all the other members of the genus *Davainea* (s. l.) bearing smaller ones. In the following table are enumerated some

Davainea species the rostellar-hooks of which are comparatively the largest ones and measure over 20μ in length:—

Species	Host	No. of rostellar-hooks	Length
*Houttuynia struthionis** (Houtt.)	*Struthio molycdophanus camelus*	1641	65–80μ[2]
Davainea (*s.l.*) *casuarii*, sp.n.	*Casuarius picticollis* ...	250	40–54μ
„ *appendiculata*, Fuhrm. ...	Unknown	130	36–43μ
„ *infrequens*, sp.n.	*Casuarius picticollis* ...	260	21–34μ
„ *fuhrmanni*, Southwell ...	*Crocopus phoenicopterus* ...	110	25–30μ
Raillietina (*Ransomia*) *undulata*, Fuhrm. ...	*Corythaeola cristata* ...	150–200	25–28μ
„ „ *campanulata*, Fuhrm.	*Perdix* sp.	40–42	27μ
„ „ *vaganda* (Baylis) ...	*Haliaëtus vocifer*	numerous	25μ
„ (*Paroniella*) *paradisea*, Fuhrm....	*Manucodia chalybeata* ...	about 100[3] about 200[4]	23μ 22μ
„ (*Skrjabinia*) *oligacantha*, Fuhrm.	*Tynamus*, sp. *Rhynchotus rufescens*	34	21–23μ
Davainea (*s.l.*) *conopophilea*, Johnston ...	?	?	23

* According to Meggitt (1921) *T. struthionis*, as described by different authors, contains more than one species, and reserving the name *Davainea struthionis* for the form firstly mentioned (without proper description) by Houltuyn and described 1885 by Parona, he separates from this latter the following species: *D. linstowi* Meggitt (1921) (= *T. struthionis* of v. Linstow (1893) and Hungerbühler (1910)) and *D. beddardi* Meggitt (1921) (= *D. struthionis* of Zilluf (1912)). The size of the rostellar-hooks is stated to be different in all the three species.

1. According to v. Linstow (1893).
2. According to Fuhrmann (1920).
3. According to Fuhrmann (1909).
4. According to Skrjabin (1914).

The four suckers are rounded in size, and exhibit a well pronounced musculature; they measure 0·4 mm. across; their border is covered with very numerous small (10μ to 13μ) hooks, which are arranged in six to ten rows. A distinct neck occurs only in stretched specimens. The segments, in most of my specimens, are much broader than long. Gravid proglottides are, apart from extremely contracted specimens, almost square.

Anatomy.

As has been mentioned above, the worms are in part greatly contracted, and their aspect is rather thick and compact. Such conditions are to be found usually in worms which possess a well

developed cortical parenchyma, subcuticular layer and cuticle. In *D. casuarii* especially the first is rather wide and exhibits a well marked longitudinal musculature. This latter consists of many more or less distinctly separated bundles of various size. The largest bundles are oval in shape, measuring about 40μ to 54μ. They are composed of thirty-five to fifty fibres of various thicknesses. Towards the subcuticular layer smaller bundles are scattered irregularly, consisting of fewer fibres, or even of but a single one. The

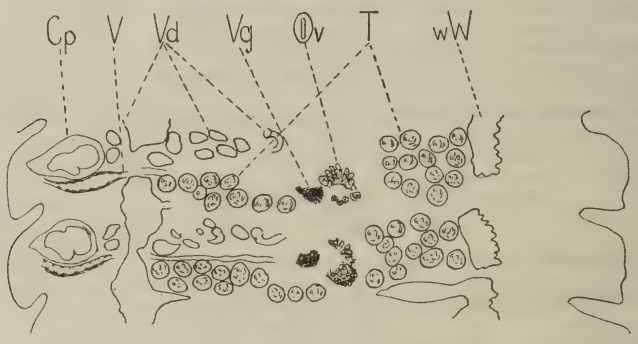

FIG. 2. *Davainea casuarii*, sp.n. Longitudinal section of two mature segments *Cp.*—cirrus pouch; *Ov.*—ovary; *T.*—testes; *wW.*—ventral excretory vessel; *V.*—vagina; *Vd.*—vas deferens; *Vg.*—vitelline gland. × 34.

transversal musculature separates very distinctly the medullary parenchyma from the cortex. Fine dorso-ventral fibres are present in both parenchyma layers, being especially well marked at the level of the transverse excretory vessel. It is worthy of note that rather large calcareous bodies are scattered in the subcuticula as well as in both parenchyma layers; they are mostly oval in shape, 10μ to 16μ in size, and deeply staining with haematoxylin.

Excretory system. The excretory system consists in main part of a single pair of very large longitudinal vessels, which are connected at the posterior border of each proglottis by a large transverse canal. Although these longitudinal vessels run justly on the transverse axis of the proglottides, having a diameter nearly equal to the depth of the medullary parenchyma, there is no doubt that they represent the ventral pair of the longitudinal vessels, for in the anterior, mostly immature proglottides, I could undoubtedly distinguish within the two large vessels two narrow, somewhat

dorsally located vessels without transverse commissures. These dorsal vessels disappear apparently in the mature segments. The wall of the excretory vessels is bordered by very minute rounded cells, which seem to be parenchyma cells.

Genital organs. The openings of the genital ducts are unilateral, the porus genitalis being situated about the centre of the lateral border of the proglottides. A small atrium genitale is present.

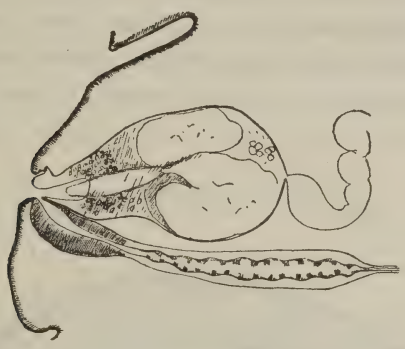

FIG. 3. *Davainea casuarii*, n.sp. Longitudinal section, showing the termination of sex ducts. × 80.

Male organs. The testes are oval or spherical in shape and 67 μ to 81 μ in diameter. They occupy the whole free space of the medulla at the sides of the female glands. On account of the structure of the vas deferens and vagina, the testes are of course more numerous on the antiporal medulla-half. Their total number amounts to nearly fifty to sixty. In the younger, and also in mature proglottides, the testes exhibit very interesting stages in the development of the spermatozoa. These stages agree in many respects with those described and drawn by Moniez (1881). The vas deferens is a rather wide, coiled tube, the coils of which occupy dorso-ventrally nearly the whole space of the poral medulla-half, displacing ventrally the wide longitudinal excretory vessel just before entering the cirrus pouch. After entering the cirrus pouch, the vas deferens forms a rather large, coiled vesicula seminalis interna, which is usually filled with spermatozoids. The cirrus is short (0·1 mm.); it is surrounded within the cirrus pouch by a dense network of very small cells, representing, perhaps, prostate cells or merely parenchyma cells.

The thick-walled cirrus pouch is pyriform, and measures 0·25 mm. in length by 0·16 mm. in breadth; it does not extend beyond the longitudinal nerve-stem, and thus does not reach at all the longitudinal excretory vessel.

Female organs. The position of the vagina, i.e., of the poral portion of the vagina in proportion to the cirrus pouch, varies according to the state of contraction of the strobila. In stretched or normally contracted specimens it lies immediately behind the cirrus pouch; in extremely contracted worms, however, it is sometimes ventral, sometimes dorsal to the cirrus pouch. The poral third of the vagina, extending from the genital atrium just beyond the poral longitudinal excretory vessel, is rather wide, darkly

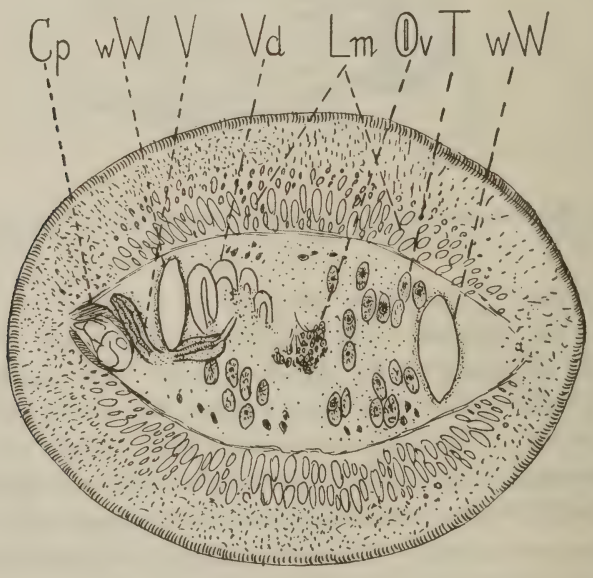

Fig. 4. *Davainea casuarii*, sp.n. Transverse section of a mature segment. *Cp.*—cirrus pouch; *Lm.*—longitudinal muscles; *Ov.*—ovary; *T.*—testes; *wW.*—ventral excretory vessel; *V.*—vagina; *Vd.*—vas deferens. × 42.

staining because of its rather muscular wall and especially on account of the presence on its inner surface of very fine hairs. A similar structure of the vagina is found in other worms, particularly in some members of the family Davaineidae, in the Tetrabothriidae, and also in certain species of the genera *Trichocephaloides*,

Monopylidium, *Octopetalum*, etc. Just before the vagina opens into the atrium genitale it bears a distinctly marked sphincter. Within the poral longitudinal excretory vessel the vagina narrows suddenly for a short distance and becomes then nearly as wide again as the vas deferens, forming some coils before reaching the ovary; its course from the longitudinal excretory vessel to the ovary is chiefly ventral, although it passes to the dorsal side of the longitudinal excretory canal, as is usually the case. A distinct receptaculum seminis is absent. The ovary is small; it is situated in the middle of the proglottides lying in longitudinal sections somewhat nearer to the posterior border of the segments and consisting of fine lobes, which radiate in all directions from the oviduct. Its diameter amounts nearly to one-fourth of the breadth

FIG. 5. *Davainea casuarii*, sp.n. Transverse section of a gravid segment. × 34.

of the medullary parenchyma. Dorso-ventrally it occupies the entire depth of the medulla, being especially well developed on the antiporal side. The vitelline gland lies quite dorsally in the median part of the medulla, where it appears as a rounded compact organ, measuring about 0·01 by 0·06 mm. A small but distinct shell-gland lies on the main trunk of the oviductus. The uterus appears very early as a rounded sac, which is situated ventrally in the middle part of the medullary parenchyma. While enlarging it becomes apparently

divided into two oval parts, both becoming confluent soon after they reach a larger size. At this stage the eggs segregate into groups of three to four eggs, which latter eventually become egg-capsules; these are mostly oval or rounded in shape; they are bordered by one to two rows of rounded, larger cells, while the two to four eggs (in transverse sections there are visible mostly two, seldom three, eggs in a capsule) are embedded in dense parenchyma containing somewhat smaller cell-elements. The egg-capsules extend beyond the longitudinal excretory vessels, and thus fill the whole parenchyma. About one hundred and eighty capsules can be counted in a transverse section; they measure 67 μ by 108 μ in diameter.

Systematic comparisons.

As already mentioned above, there has been only one species of the genus *Davainea* hitherto known from birds belonging to the Casuariformes, viz., *Davainea* (s. l.) *australis* (Krabbe). *D. casuarii*, sp. n., differs from this cestode in many respects, but especially in the shape and size of the scolex and the rostellar-hooks. Comparing this new cestode with other members of the genus *Davainea* (s. l.), I find that it agrees in general with the type known in *Davainea*. There is, however, no doubt that it bears some characters which are to a certain degree rare or unusual in this genus; such features are the considerable size of the rostellar-hooks, the absence of one pair of excretory vessels and the well-developed longitudinal musculature. Owing to these peculiarities it seems that there exists a certain relationship between *D. casuarii* and the genus *Porogynia*, Railliet et Henry (= *Polycoelia*, Fuhrm.). The arrangement of the genital glands, however, which in our cestode is of the usual type of *Davainea*, does not allow it to be assigned to *Porogynia*, which latter, moreover, bears three rows of rostellar-hooks on the scolex. On the other hand, it is not possible to place this cestode into one of the genera recently established by Fuhrmann (1920), mainly because of the above-mentioned unusual features. Among all these new genera it is to the large genus *Raillietina*, Fuhrm., sub-genus *Ransomia*, Fuhrm., that our cestode should be assigned, if we do not consider the above-mentioned characters to

be of systematic value, warranting the creation of a new genus or sub-genus for it. For myself, I am inclined to believe that the establishment of a new sub-genus might be justified.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

DAVAINEA (s. l.) *INFREQUENS*, sp. n.

Host: *Casuarius picticollis*, Sclat.

Locality: Sattelberg.

Only a few specimens of this cestode were found in the same host as *D. casuarii*. The worms are much smaller and narrower than the former species. Unfortunately there were only incomplete individuals available. From two fragments, which belong apparently to one another, one can estimate the total length of the strobila at about 80 mm. by a greatest breadth of 1·2 mm. in the posterior third. The scolex is globular, measuring 0·5 mm. across. It exhibits a fairly well developed rostellum of 0·25 mm. in diameter, bearing a double row of typical hammer-shaped hooks. Their number is about two hundred and sixty. They measure in the anterior row 27μ to 34μ, in the posterior row 21μ to 25μ in length. The suckers are spherical, their diameter being 0·13 mm. They are bordered by four to six rows of hooks, 10μ to 15μ in length. There is a well marked neck measuring about 2 mm. in length. The proglottides are broader than long.

Anatomy.

The internal anatomy of the worms exhibits the usual characters of the genus *Davainea*; the structures seen in transverse and longitudinal sections were much like those found, especially in some *Davainea* species of Psittaciformes.

Excretory system. There is only one pair of large longitudinal vessels, lying usually nearer to the ventral side.

Musculature. The longitudinal muscles are well developed; they are composed of an internal layer consisting of about sixty

large oval bundles, and by a distinctly separated external layer, which exhibits two rings of very small bundles consisting of at most two to three fibres. Similar arrangement of the longitudinal musculature occurs also in *D. spiralis*, Baczynska (1914).

Oval calcareous bodies are present, especially in the cortical parenchyma.

Genital organs. It seems that the openings of the sex-ducts are unilateral, lying on the left side; in one segment (of about twenty), however, I found the opening on the right. The thick-walled cirrus pouch is 0·18 to 0·2 mm. in length, 0·06 mm. in breadth; it extends to the poral longitudinal excretory vessel. The cirrus is short, rather thick at its anterior end and covered with many spine-like elements. It bears retractor muscles which radiate in all directions to the wall of the cirrus pouch. Within this organ there is an oval vesicula seminalis interna measuring 0·054 mm. in length. The vas deferens forms many large coils in its course towards the middle of the medulla. The testes are about nine to twelve in number, lying not only at both sides of the female glands, but also in the median line of the segments. They measure 0·05 mm. in diameter.

The vagina lies behind the cirrus pouch. Its structure is the same as, e.g., in *D. aruensis*, Fuhrm. (1911) or in *D. allomyodes*, Kotlán (1921). In the middle of the segments, shortly before reaching the ovary, it forms a small spindle-shaped receptaculum seminis. The bilobed ovary, when fully developed, is about 0·2 mm. in breadth; it lies in the middle of the segments. Behind the ovary is situated the compact vitelline gland, which is about 0·08 mm. broad.

Gravid proglottides are not available, and I am, therefore, unable to give a complete description of this worm. The above noted characters are, however, I believe, sufficient to distinguish this form from other members of the genus *Davainea*, of which the following must be considered mostly on account of the similar size of the rostellar-hooks:—*D. fuhrmanni*, Southwell (1922), *Raillietina* (*Ransomia*) *undulata*, Fuhrm. (1909), *R.* (*R.*) *campanulata*, Fuhrm. (1909), and *R.* (*R.*) *vaganda*, Baylis (1919).

The new worm in question seems in every way to be closely related to *D. fuhrmanni*. Comparing, however, the characteristic features of our worm with those of *D. fuhrmanni*, described in

detail by Southwell (1922), I conclude that there are some differences which do not permit the two species to be united. Such are:—

1. The smaller number of the rostellar-hooks in *D. fuhrmanni*.
2. Larger and, in some respects, better preserved material would perhaps show that the genital openings are irregularly alternate in *D. infrequens*.
3. It seems that the cirrus pouch in *D. infrequens* is longer and rather narrower in size.
4. There is no mention in the description of *D. fuhrmanni* of the distinct vesicula seminalis interna.
5. No mention is made of the presence in *D. fuhrmanni* of retractor muscles of the cirrus.
6. The prostate-cells surrounding the coils of the vas deferens are inconspicuous in *D. infrequens*.
7. Finally, it seems improbable that one and the same species of worm should be found in Columbiform and Casuariform birds.

The three other species mentioned above differ from *D. infrequens* in the number of the rostellar-hooks and in other anatomical characters.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

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AVIAN CESTODES FROM NEW GUINEA

III. CESTODES FROM GALLIFORMES

BY

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Only one representative of the bird-group Galliformes has been examined for parasites, viz., *Megapodius brunneiventris*, Mey. The tapeworms which were found in the gut of this bird belong to three distinct species of the genus *Dilepis*, Weinl; one of these is smaller and narrower than the two other species, and is, therefore, easy to separate from these latter. It requires, however, a careful examination to be able to distinguish the two other species, the scolex and strobila of which are quite similar to one another. All three species are, I believe, undescribed; the genus *Dilepis*, so far as I am aware, has not yet been recorded from Galliform birds.

DILEPIS YORKEI, sp. n.

Host: *Megapodius brunneiventris*, Mey.

Locality: Friedrich-Wilhelmshafen.

This is the smallest of the three species mentioned above; fully matured specimens measure 15 to 20 mm. in length. The scolex is

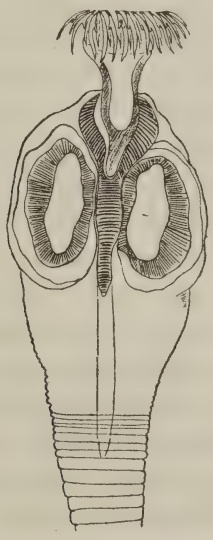

FIG. 1 *Dilepis yorkei*, sp.n. Scolex. × 50.

very well developed, it is nearly as long (0·7 mm.) as broad (0·5 to 0·6 mm.). It bears a rather powerful rostellum of conical shape and of about 0·5 mm. in length. This rostellum, when retracted, is surrounded by a double muscular rostellar sac of about 0·7 mm. in length. On the anterior end of the rostellum there is a button-like thickening of nearly 0·17 mm. in diameter, bearing fifty to fifty-two large hooks, which are arranged in a double row.

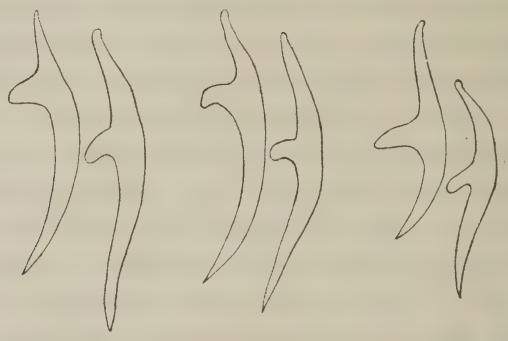

Fig. 2. Hooks from the rostellum. A.—*D. yorkei*; B.—*D. leptophallus*; C.—*D. horvâthi*. × 230.

They measure in the anterior row 135μ, in the posterior row 148μ to 151μ. The suckers are oval in shape and measure 0·42 to 0·44 by 0·25 to 0·30 mm. in diameter.

Behind the scolex there is a very short unsegmented portion, which is usually broader than the segments of the anterior half of the worm. The strobila of a fully developed specimen consists of about one hundred and twenty to one hundred and fifty segments; these are, as a rule, broader than long, except in macerated specimens. Mature segments are 0·2 to 0·4 mm. in breadth and 0·05 to 0·1 mm. in length. The greatest breadth (0·3 to 0·5 mm.) is attained in the last fourth of the strobila with gravid segments.

Anatomy.

Body-wall and parenchyma. The cuticle, as in other similarly delicate cestodes, is rather thin and not at all compact. The subcuticular cells are fairly well developed and arranged into two or three rows. The body-parenchyma is of peculiar structure, consisting of a loosely arranged reticulum with rather poorly scattered cell-elements. Calcareous bodies were not found.

Musculature. The somewhat denser cortex is separated from the very loose medulla by the longitudinal muscles, which are arranged in two rings, each being composed of a row of small inconspicuous muscle-bundles. Inside of the interior row there is apparently a very poorly developed transverse musculature. Dorso-ventral muscle-fibres were not seen.

Excretory system. In the anterior two-thirds of the strobila there exist two longitudinal vessels on each side of the segments, of which the ventral is slightly larger than the dorsal. In segments in which the uterus reached a more considerable extent, only one pair of longitudinal vessels can be seen. Transverse commissures were not observed.

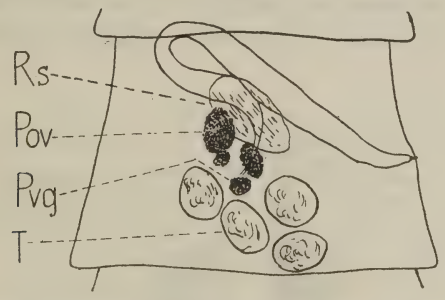

FIG. 3. *D. yorkei*, sp.n. Younger segment showing mature male organs and primordial female glands. *Pov.*—primordial ovary; *Pvg.*—primordial vitelline gland; *Rs.*—receptaculum seminis; *T.*—testes. ×170.

Genital organs. The first indication of the sex-organs appears already in the first distinct segments. As in many other cestodes, the male organs are markedly more advanced in development than the female organs. The cirrus pouch attains its largest size by the twentieth segment; then follow the testes, which, however, disappear about the sixtieth to seventieth segment, while the female glands, which also appear rather early, reach full maturity after and about the eightieth segment. Here also the uterus appears, and grows rapidly to a considerable size.

The genital-openings are unilateral, being situated about the middle of the lateral border of the proglottides.

Male organs. The cirrus pouch, compared with the size of the proglottides, is a large tube of 0·18 to 0·2 mm. length and 0·021 to 0·027 mm. greatest breadth. Its position varies according to the

state of contraction of the worm. In somewhat longer segments it is directed obliquely to the anterior end of the segment. After narrowing for a short distance it is continued by a very wide vas deferens, which, forming one or two large coils, runs to the posterior half of the segment. The cirrus seems to be a fairly slender canal, which on its anterior end is apparently covered with minute spines.

There are only four testes in each segment, situated in the middle of the posterior third; they are 37μ by 27μ in diameter.

Female organs. The vagina, a fairly short and narrow canal, runs dorsally to the cirrus pouch. It forms a large (about 0·08 mm.) receptaculum seminis, which lies immediately within the dorsal longitudinal musculature extending to, or but little beyond, the middle of the proglottis. The ovary exhibits a peculiar structure,

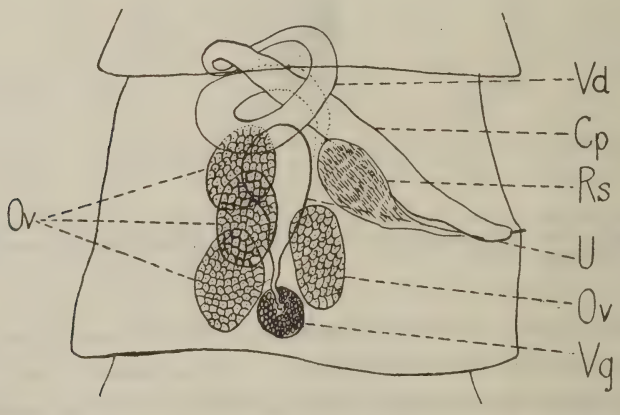

Fig. 4. *D. yorkei*, sp.n. Older segment showing mature female organs. *Cp.*—cirrus pouch; *Ov.*—ovary; *Rs.*—receptaculum seminis; *U.*—uterus; *Vg.*—vittelline gland; *Vd.*—vas deferens. ×170.

which, though slightly modified, is characteristic of the two other species also. In the present case it consists of four nearly equal bodies, which are rounded or mostly oval in shape, measuring about 54μ to 67μ by 40μ. One of these ovarial sacs is situated in the poral half, while the three others lie antiporal, i.e., two of them ventral and one somewhat dorsal. Each ovarial sac sends out a thin-walled canal; these unite into a larger, very short oviduct. On the main trunk of the oviduct lies a rounded shell-gland.

A globular vitelline gland of 29μ diameter is seen in the mid-line towards the posterior margin of the segments.

The young uterus is a thin-walled sac, which lies ventrally in the anterior half of the segments. Growing to a more considerable size, its walls become more distinct; in this stage the female glands disappear suddenly, the whole medulla being occupied by the uterus. In the two or three last segments, however, the wall of the uterus atrophies, the ripe ova filling up the whole space of the proglottides. The rounded ova measure 54μ in diameter.

I have named this species in honour of Prof. Warrington Yorke, of the University of Liverpool.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

DILEPIS LEPTOPHALLUS, sp. n.

Host: *Megapodius brunnieventris*, Mey.

Locality: Friedrich-Wilhelmshafen.

The longest worms, when fully developed, measure 80 mm. in length. The scolex is rather similar to that of the former species, its diameter being 0·68 mm. The rostellum is, in its main features, like that of *D. yorkei*, measuring 0·64 to 0·76 mm. in length. It bears on its anterior, knob-like end fifty-two hooks arranged in a double row, those in the anterior row being slightly smaller in size (121μ to 126μ) than those in the posterior row (135μ to 143μ). The four suckers are rounded in shape, measuring 0·3 to 0·34 mm. in diameter.

Segmentation begins just behind the scolex. The proglottides are, as a rule, broader than long. The ratio of the length to the breadth varies according to the different stages of contraction. This ratio is in my specimens mostly as 1 : 4-6, so far as concerns the proglottides of the anterior half of the strobila; backwards (caudad) the length increases slightly, the ratio becoming as 1 : 3. The anterior end of the proglottides is usually much narrower than the posterior, which in most specimens shows a distinct thickening.

There is a very well pronounced overlapping, especially in the posterior half of the strobila. Gravid segments are about 2·5 mm. broad and 1·5 mm. long, and exhibit a considerable thickness.

ANATOMY.

Musculature. The longitudinal muscles consist of bundles, which are arranged in two layers; the internal layer exhibits twenty-six to thirty, the external sixty-six to seventy, mostly oval bundles. The transversal musculature is very poorly developed; mostly it seems entirely absent. The dorso-ventral muscle-fibres are likewise but faintly distinguishable.

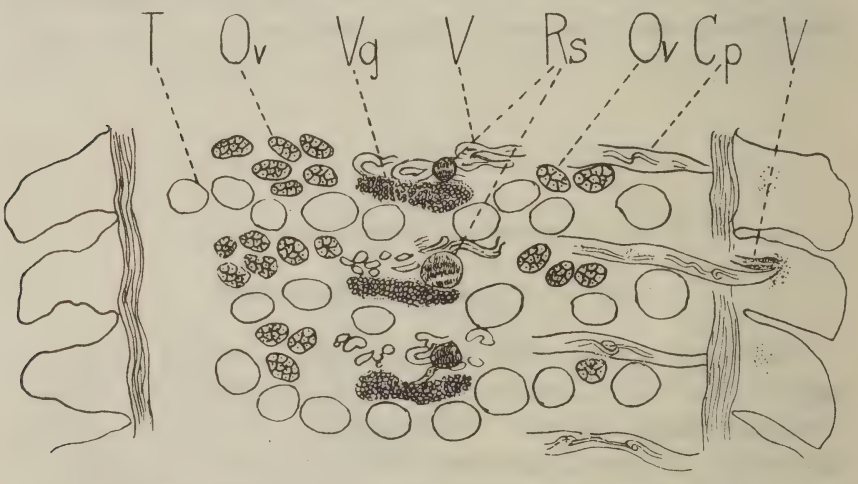

FIG. 5. *D. leptophallus*, sp.n. Longitudinal section of three mature segments. *Cp.*—cirrus pouch; *Ov.*—ovary; *Rs.*—receptaculum seminis; *T.*, testes; *V.*—vagina; *Vg.*—vitelline gland. × 100.

It is worth noting that in none of the specimens examined could any trace of calcareous bodies be detected.

Excretory system. This consists of two pairs of longitudinal vessels, of which only the two wide ventral ones form a transverse canal, this latter being approximately as wide as the main ventral vessels.

Genital organs. The openings of the genital ducts are unilateral and lie on the posterior third of the lateral border. The atrium genitale is marked off by a dense network of very small cells of rounded or oval shape.

Male organs. The cirrus pouch consists of a very long narrow tube of about 0·68 mm. length and 0·02 mm. breadth. Its position and course depend essentially on the state of contraction of the proglottides, and especially on the progress in development of the genital organs. According to this it runs in normally contracted mature segments from the genital pore to the excretory vessels on the transverse axis, and then turns dorsally, extending a little beyond the middle of the segments. In longer proglottides exhibiting fully developed genital glands, the course of the cirrus pouch is very different from that described above, as (seen in optical longitudinal section) it runs from about the genital pore to the anterior third of the segment parallel to the lateral border, and then turns abruptly to the median part. The cirrus pouch possesses long retractor muscles, which extend beyond the antiporal ventral

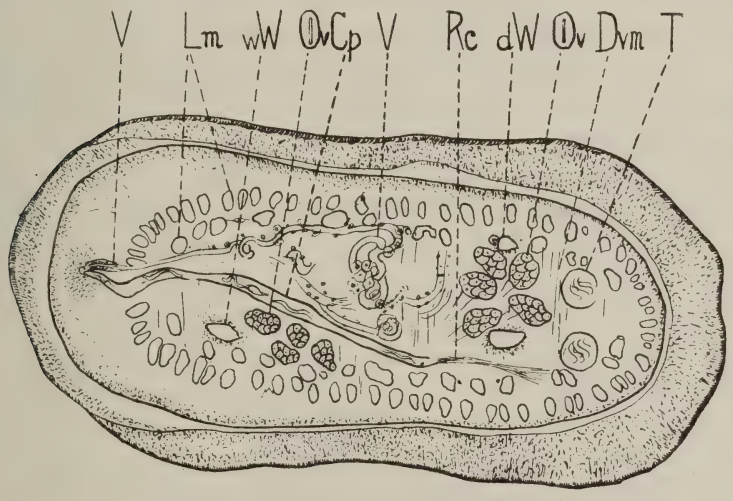

Fig. 6. *D. leptophallus*, sp.n. Transverse section of a mature segment. *Cp.*—cirrus pouch; *Dvm.*—dorsoventral muscle fibres; *Lm.*—longitudinal muscles; *Ov.*—ovary; *Rc.*—retractor muscles of the cirrus pouch; *T.*—testes; *dW.*—dorsal excretory vessel; *vW.*—ventral excretory vessel; *V.*—vagina. × 80.

vessel. There is a coiled vas deferens, the coils of which lie in transverse section mainly dorsal, and in a somewhat higher plane than those of the vagina. The cirrus is a very long and slender (8 μ) canal, forming usually many coils within the cirrus pouch; in some specimens, in which it was extruded, I could observe that at least on the anterior half it is covered with very minute spines.

Female organs. The vagina is a strikingly long duct which runs from the atrium genitale to the mid-part of the medulla, mainly just within the internal layer of the dorsal longitudinal muscles; it forms very large coils, which fill dorso-ventrally the entire central space of the medullary layer. The walls of the vagina are generally thin and covered with rounded cells; immediately before entering the small atrium genitale its walls present a slight sphincter-like thickening. Just in front of the shell-gland there is a rather large rounded receptaculum seminis. The ovary is composed of poral and antiporal lobes, each consisting of distinctly separated groups of acini, the poral lobe having about five groups and the antiporal about ten. The acini are all rather similar in size, measuring about 43μ across. Each group sends out a thin-walled, narrow canal, all of which, running into a larger one, form a distinct ovarian bridge connecting the two groups of the ovary; from the mid-part of this bridge there arises, at first somewhat dorsally directed, a rather wide oviduct. A similar structure of the ovary exists also in other cestodes, of which the following may here be mentioned:— *Choanotaenia porosa* (Rud.) (see Cohn, 1901), *Ch. gongyla*, Cohn (1901), *Anomotaenia platyrhyncha* (Krabbe), *A. microrhyncha* (Krabbe), and *Ophryocotyle herodiae*, Fuhrm. (1909). A compact, somewhat bean-like vitelline gland lies in the middle of the medulla; it measures 0·14 mm. At the junction of the oviduct and vitelline duct there is a distinct shell-gland of rounded shape.

The uterus appears at first as a rounded, thin-walled sac between both groups of the ovary. It then grows very rapidly, sending out oval diverticula laterally, and usually beyond the excretory vessels as well. All of these sacs then flow together to form a larger one, which at this point has already a more distinct cellular wall. It is an interesting feature that the testes and the receptaculum seminis still persist for a rather long time, the uterus having already occupied transversely almost the whole of the proglottis. In the last few segments the wall of the uterus atrophies, and they are entirely occupied by the ova. The ripe ova measure 64μ by 54μ in diameter.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

DILEPIS HORVÁTHI, sp. n.

Host: *Megapodius brunneiventris*, Mey.

Locality: Friedrich-Wilhelmshafen.

Among the cestodes collected from this bird I found only a few chains belonging to this new species. With the naked eye it is not easy to distinguish them from *D. leptophallus*. The worms are apparently somewhat shorter than the latter species, the longest specimens measuring 50 mm. The scolex closely resembles in its shape and size that of the former species, being 0·8 mm. in width. The rostellum is still larger, and when retracted it extends with its posterior end to 0·7 mm. behind the posterior border of the suckers. On the anterior knob-like thickening there are fifty-two hooks arranged in a double row; there is but little difference in size between the hooks of the two rows. I found them to be 99μ in length in the anterior, and 102μ in the posterior row. The shape, especially that of the anterior hooks, slightly differs from the type shown in the two other species. The suckers are rounded in shape and measure 0·3 mm. in diameter. There is no neck, except in stretched specimens; the segmentation begins a short distance

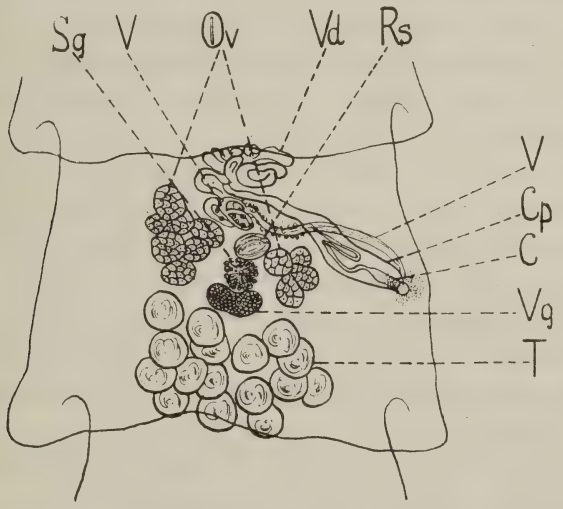

FIG. 7. *D. horváthi*, sp.n. Mature segment. *C.*—cirrus; *Cp.*—cirrus pouch; *Ov.*—ovary; *Rs.*—receptaculum seminis; *Sg.*—shell gland; *T.*—testes; *V.*—vagina; *Vd.*—vas deferens; *Vg.*—vitelline gland. × 80.

behind the suckers. The segments in my few chains are usually broader than long, and show otherwise the same features as in *D. leptophallus*. The greatest breadth found in the posterior part of the strobila is 1·5 mm.

Owing to the small number of specimens available belonging to this species, I omitted to sacrifice a chain for the purpose of cutting sections. The following description of the arrangement of the sexual organs is, therefore, based mainly upon worms stained as a whole with boraxcarmin and mounted in balsam.

The genital pores are unilateral, and lie about the middle of the lateral border. It seems that the atrium genitale is of the same extent as in *D. leptophallus*.

The cirrus pouch is a somewhat shorter but wider tube than it is in the former species, measuring about 0·2 mm. in length and 0·04 mm. in breadth; it is usually directed with its long axis obliquely forwards. Within the cirrus pouch is found the rather long cirrus, which at its extremity is distinctly thickened and covered with minute spines. The vas deferens forms many coils, which lie mainly in the middle of the anterior end of the proglottides. The testes lie behind the female organs; it seems that they are less numerous (about fifteen to seventeen) than in the former worm; they measure 54μ to 64μ.

The vagina rises anterior to the cirrus pouch; it crosses the posterior end of this organ and then forms apparently as many coils as that of *D. leptophallus*. A rounded receptaculum seminis is similarly present.

The ovary exhibits the same peculiarities as in *D. leptophallus*. If any difference exists in the structure of this organ in both forms, it might perhaps lie in the somewhat fewer number of the ovarian lobes on both the poral and antiporal side.

The vitelline-gland is similar in shape and size to that of the former species.

As gravid segments were not at hand, I am unable to give a suitable description of the uterus and the ripe ova.

The main features which distinguish this species from *D. leptophallus* are:—

1. The shape and size of the rostellar-hooks.
2. The shape and size of the cirrus pouch.

I have named this species in honour of Dr. G. Horváth, Director of the Zoological Department of the Hungarian National Museum in Budapest.

The type specimen is in the Parasitological Museum of the Royal Hungarian Veterinary College, Budapest.

Among the known representatives of the genus *Dilepis*, there is, I believe, none which exhibits a closer resemblance, so far as the above-mentioned peculiarities are concerned. The type of the hooks might in some respects be likened to those of *D. macrocephala*, Fuhrm. (1908), the scolex and rostellum of which are likewise strong.

All the three species described above are closely related to each other. This is proved by the following features:—

1. The structure of the scolex and its integrate parts (especially the rostellum and hooks).
2. The structure of the male organs, viz., the large cirrus pouch, the reduced number of testes.
3. The structure of the female organs in general and mainly of the ovary.

In philogenetic respects it seems doubtless that the three forms, but particularly *D. yorkei*, are very old representatives of the genus *Dilepis*, and might be perhaps interpreted as a distinct group within this genus.

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AN ANOPHELES OF THE *MYZORHYNCHUS* GROUP (*Anopheles amazonicus* SP.N.) FROM SOUTH AMERICA

BY

LIEUT.-COL. S. R. CHRISTOPHERS, C.I.E., I.M.S.

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PLATE IV

Among some material at the Liverpool School of Tropical Medicine, which owing to the kindness of Professor R. Newstead, F.R.S., I was able to examine when home on leave, I was fortunate enough to find a specimen of an anopheline brought by Dr. A. A. Clark from the River Amazon, which not only seemed to be new, but which appeared to be the first instance of an undoubted *Myzorhynchus*, using this term in its restricted sense, recorded from South America, or, indeed, from the New World. On looking up material previously brought by Dr. Clark from this region, Professor Newstead was able to find two other specimens of the same species not quite in such good preservation. With Professor Newstead's kind permission, I give below a description of this species under the name *A. amazonicus*. All three specimens were females, the first mentioned being selected as the type and so labelled in the Liverpool School collection.

A. amazonicus closely resembles the Old World species of the group *Myzorhynchus*, and it possesses the ventral abdominal scale tuft on the penultimate abdominal segment which Theobald used to define the genus *Myzorhynchus* when he created it, though it is now known that this character is not present in all the species of the group. In one character, however, *A. amazonicus* approaches the *Arribalzagia* group, which normally, so to speak, represents *Myzorhynchus* in South and Central America. The character referred to is a kink or bend in the costa at the subcostal junction associated with one or more small accessory dark spots in this

position. From the description it will be seen that *A. amazonicus* possesses this feature, though it shows nothing of the other more salient *Arribalzagia* characters such as inflated wing scales, eye-spots on the thorax, abdominal scaling, etc.

A very marked specific feature of *A. amazonicus* is the great length of the anterior forked cell, which measures one-third of the wing length and extends so far inwards that the bifurcation is at the level of the junction of the subcosta with the costa.

Anopheles (*Anopheles*) *amazonicus*, sp. n.

Diagnostic Points.

An easily identified species characterised by:—

(1) The wings with pale interruptions on the costa.
(2) The palps shaggy and without definite bands.
(3) The hind tarsi dark.
(4) The femora and tibiae unicolorous.
(5) The bifurcation of the second longitudinal vein at the same level as the junction of the subcosta with the costa.
(6) A ventral scale tuft on the penultimate abdominal segment.

Detailed Description.

♀. A largish dark anopheline of *Myzorhynchus* appearance; general coloration rather rusty black. Length of wing, 4·4 mm.

Antennae with the basal segment dark, free from scales; the second segment with a small tuft of pale and darkish scales on inner aspect; remaining segments free from scales. *Palpi* with the segments, commencing with the rudimentary basal one, measuring respectively 6, 21, 35·5, 21·5 and 16 per cent. of the whole organ. Palpal index (measured from unmounted specimen) 0·7. General appearance of palpi as in *A. umbrosus*, Theo.; densely covered with black erect or semi-erect scales almost to the apex, but with these longer on the basal rudimentary and succeeding segment; apex dark and organ without obvious pale bands, though one or two light scales are present at the apices of segments three and four difficult to see except in certain lights. *Labium* black scaled, the

scales somewhat erect over basal half; labellae darkish. *Clypeus* dark, bare. *Head* with the frons and vertex with small very narrow white scales, less prolonged than usual. The pale area smaller in extent than usual; including the area of narrow scales in front and about the same extent of the ordinary upright scales behind this. Occiput with dark erect truncate scales of ordinary anopheline type, extending below level of neck. Some broad white scales beneath, between the eyes, gular chaetae black.

Prothoracic lobes with dense tufts of black erect scales and numerous chaetae. *Prosternal hairs* about four. *Mesonotum* of uniform coloration, dull brown, the bare spaces, etc., not conspicuous; chaetae inconspicuous and presence of median series doubtful. The surface clothed with light coloured hairs, scantily but fairly uniformly distributed over the dorsum, including the fossae. Anterior promontory with a smallish area medially of long curved, pale scales, not forming a conspicuous feature; laterally, and extending about half way to the lateral angular process of the mesonotum, are rather conspicuous erect pale spatulate scales. *Scutellum* with about twenty-four large hairs and a second line of two additional hairs on each lateral lobe; scattered smaller impressions (scales or hairs) medially. *Spiracular hairs* about two. *Pre-alar hairs* about eight.

Wings with the length 4·44 mm. and the greatest breadth 1·05 mm. Base to subcostal junction 0·67, anterior forked cell 0·33, posterior forked cell 0·19 of the length of the whole wing. Forked cell index 1·8. The anterior forked cell unusually long, and the bifurcation of the second longitudinal vein so far towards the base of the wing that it is on a level with the junction of the subcosta with the costa.

The wing markings, as a whole, are rather diffuse, the pale areas not being very distinct, whilst there is an admixture in places of pale and dark scales. Costa mainly dark, but with the following pale areas: a minute one near base, a well marked one at about the junction of the inner with the middle third of the wing length; a comparatively large one just internal to the subcostal junction, a somewhat smaller one actually at the subcostal junction and one at the apex of the wing not quite reaching to the point of junction of the first longitudinal with the wing margin. The most

characteristic feature of the costal markings is the presence of the small accessory dark spot, involving the costa only, which lies between the two pale areas in the region of the subcostal junction. In this position there is also seen a slight but distinct bend or kink in the costa, as in species of *Arribalzagia*. The *first vein* is marked as the costa, but with additional pale areas at the base and at the accessory sector. The *second vein* has the stem mainly pale scaled, with dark scales at the cross-vein and just distal to its origin; the upper branch has a small pale spot just external to the middle, and the lower branch an indistinct one somewhat internal to this; the branches are also pale where they join the wing margin, though the fringe itself here is dark. The *third vein* is mainly dark, but has light scaled areas, separated by a conspicuous small dark spot, on its basal portion. The *fourth vein* stem is dark, with white scaled areas near the base and proximal to the cross-vein. The branches are mainly dark with pale scales forming one (or two) small spots on the upper branch. The *fifth vein* has the stem with mixed dark and pale scales, the anterior branch with pale patches near the base and beyond the cross-vein, the posterior branch with pale (mixed with dark) scales on its proximal and dark scales on its distal half. The *sixth vein* has alternate dark and light portions (four pale and four dark areas). The *fringe* is dark from the apical costal spot to the space between the veins 3 and 4·1 where there is a light spot. There is another somewhat indefinite pale spot between 4·2 and 5·1. The remainder of the wing fringe is too rubbed in all the specimens for description.

Except for the spots on the costa and on vein 2·1, the scales of the under surface are all dark. The wing membrane is stained, but is lighter at some of the pale scaled areas.

The scaling of the wing shows the normal arrangement, but the truncated squames of the median series are very inconspicuous owing to the development of the laterals, whilst these latter and the plume scales of the reverse side of the veins approach each other in character so closely that they are scarcely to be distinguished. The general effect is a heavy scaling with rather uniform large obovate scales. The squames show from seven to nine striations, the laterals ten to eleven, and the plumes usually nine striations, but some slightly broader plumes are present on the fourth vein (upper

surface), where they may show as many as twelve striations. This is the position where the large inflated scales of *Arribalzagia* occur.

The *coxae*, in the case of the anterior pair, have black scales basally and anteriorly, also posteriorly and apically. The middle and posterior pair appear devoid of scales. The anterior *trochanters* with black and white scales, the middle and posterior apparently devoid of scales. *Femora* of the anterior pair moderately dilated in inner half. The femora and tibiae of all the legs without definite markings, except that there is a lightish triangular spot on the mid-tibia apically. Tarsal segments of all the legs dark, unicolorous, but with the apices of segments one, two and three narrowly pale, four and five being dark.

The abdomen with hairs only, except ventrally. Cerci with hairs only. Ventral surface with hairs, except medially, where on segments four to seven, somewhat nearer the posterior than the anterior border of the segment, are small patches of white scales, the number of scales increasing up to the patch on the seventh segment. On the seventh segment, posterior to the white scales, is a prominent projecting tuft of black scales.

HABITAT, etc. The specimens were collected by Dr. A. A. Clark on his journeys up and down the Amazon. The type was labelled 'A. A. Clark, River Amazon, June, 1915.'

A. amazonicus is distinguished from *A. vestitipennis*, Dyar and Knab, to which it has some resemblance, by the absence of speckling of the femora and tibiae, by the tarsal markings and by differences in the wing markings. It is distinguished from *A. crucians*, Wied., by the costal and palpal markings, from the Myzorhynchellas by the uniform colour of the hind tarsi, and from *A. peryassui*, Dyar and Knab, by the absence of eye-spots on the thorax, etc.

The only species about which some doubt must remain is *A. mattogrossensis*, Lutz and Neiva. The description of *A. mattogrossensis* given by Lutz and Neiva (1911) corresponds in a number of respects with the species now described. But the wings are described as 'rather dark, especially on the costa, where there are two spots lighter in colour, greyish yellow; there is a band, whitish-yellow, transversal and sub-apical, formed by a group of cream-coloured scales; there are others distributed in a somewhat irregular manner upon the longitudinal veins, scarcely distinguishable by the

naked eye.'* As there are three quite distinct spots on the costa of *A. amazonicus*, the correspondence here would not seem to hold good. The description also says nothing about a ventral tuft on the penultimate segment, though the ventral surface of the abdomen is described as 'having traces of elongate scales, narrow and rather long.'† In *A. amazonicus* the scales, other than those forming the tuft, are few in number, and would scarcely be described in the words used in the description of *A. mattogrossensis*. Unfortunately the description given of *A. mattogrossensis* is rather meagre, and I am unaware of any other reference to this species giving any further particulars, whilst the type is presumably in South America.

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Lutz and Neiva (1911). Notas Dipterolojicas. *Mem. Inst. Osw. Cruz.*, Vol. III, p. 297.

* The original passage reads, 'Azas bastante escuras, principalmente na costa, onde ha duas manchas de côr mais clara, amarelo-pardacenta; ha um risco branco-amarelado transversal e subapical, formado por um agrupamento de escamas de côr *creme;* ha outras, distribuidas de modo um tanto irregular, sobre as nervuras lonjitudinais, que apenas se distinguem a olho nú.'

† 'na ventral ha vestijios de escamas alongadas, estreitas e pouco compridas.'

EXPLANATION OF PLATE IV

Fig. 1. Camera-lucida drawing of wing of *A. amazonicus.* The scales are not shown, but the positions of the dark and pale scaled areas on the veins are indicated by shading.

Fig. 2. Truncated squames of the median series (obverse scaling).
a. Costa internal to subcostal junction.
b. First longitudinal, basal portion.
c. do. at level of subcostal junction.
d. Stem of vein 5.

Fig. 3. Lateral squames (obverse scaling).
a, *b*, *c.* As in fig. 2.

Fig. 4. Scales *in situ* on anterior branch of vein 5.
m. Median.
l. Lateral.
pl. Plume scales of reverse side of vein seen through the wing membrane. Vein 5 is a normal vein, *i.e.*, the squame scales are uppermost.

Fig. 5. Plume scales of reverse aspect of veins.
a. Costa external to subcostal junction.
d. Stem of vein 5.
e. Stem of vein 4, upper surface. Vein 4 is a reverse vein, *i.e.*, the squames are beneath and the plume scales uppermost.

Fig. 6. Ventral view of terminal portion of abdomen, showing scale tuft.

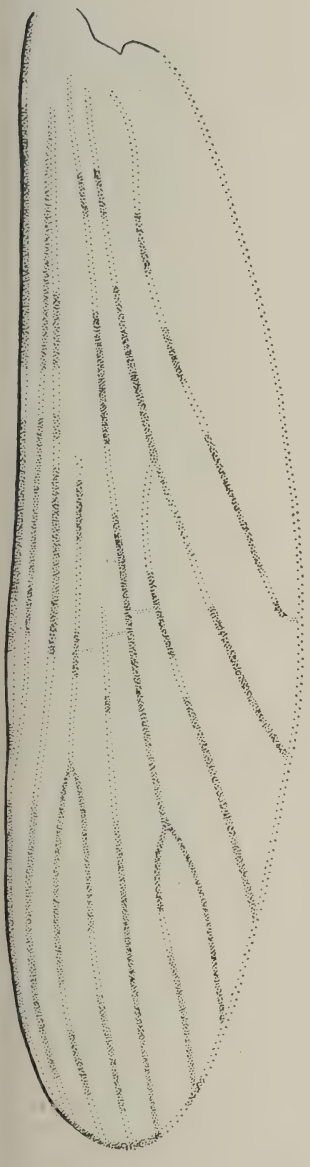

Fig. 1

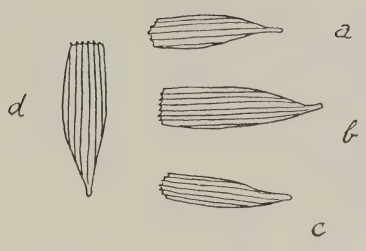

Fig. 2

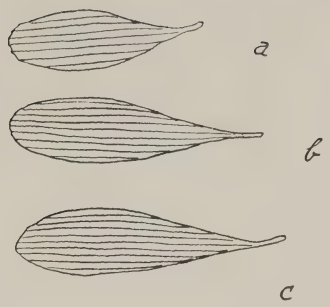

Fig. 3

Fig. 4

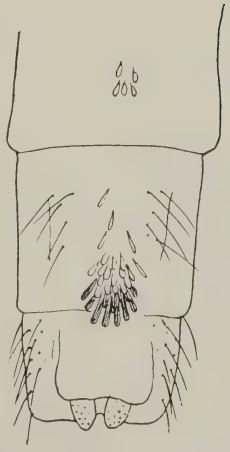

Fig. 6

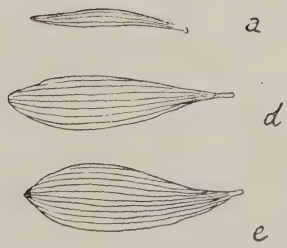

Fig. 5

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THE ETIOLOGY OF BLACKWATER FEVER

BY

B. BLACKLOCK

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown*)

(*Received for publication* 5 *February*, 1923)

Marchiafava and Bignami (1894), in referring to malarial poisoning, say: 'We may mention those morbid states which are developed after the malarial (parasitic) infection has passed away; for instance, the post-malarial fevers, the delirium, the post-malarial haemoglobinuria.' Mannaberg (1894) emphasises the fact that Kelsch and Kiener proved that in every severe case of malaria, even in every malarial cachexia, haemoglobinuria may be observed, and calls attention to the effects of lesions of the renal epithelium as pointed out by Bignami. These observers recognised haemoglobinuria as a relatively common complication or sequela of malaria, and it is of interest that they recognised it as a fact that, when haemoglobinuria develops after malaria, the parasites have disappeared from the peripheral blood.

Later quinine was added as a supplementary cause of the attacks of haemoglobinuria. Of the numerous more recent theories of the cause of blackwater fever with which we are chiefly concerned here, the first is Manson's, who stated that blackwater fever is a disease by itself, separate from and not dependent upon malaria; the second, which is an advance on this, claims for blackwater fever that it is produced by a living organism. Further suggestions, still largely in the realm of speculation, have been made by which this or that form of parasite is stated to be the cause of blackwater fever. Some of these parasitic theories we shall have the opportunity of mentioning later.

Although it is thirty years ago since Manson promulgated the theory that blackwater fever is a disease entity, and although numerous observers since have attributed to different organisms the

credit of being the cause of it, the specific parasite which gives rise to blackwater fever is still undiscovered. The older malaria, malaria-quinine and similar theories have suffered much at the hands of critics, but to fill their place little of definite value has been produced. The tendency has been to admit that while haemoglobinuria occurs as the result of malaria, and quinine and other drugs, yet apart from these haemoglobinurias, and separable from them even when, in the tropics, they occur in chronic malaria cases who have been taking quinine, there is a definite recognisable condition of haemoglobinuria which constitutes the main sign of blackwater fever.

Castellani and Chalmers (1919) differentiate the haemoglobinurias which may occur in the tropics into three groups: Symptomatic, Toxic and Specific. Under symptomatic they put haemoglobinuria occurring in the course of malaria, Raynaud's disease, acute specific fevers, and after severe burns. The toxic group includes haemoglobinuria resulting from the administration of quinine and its salts, chlorate of potash, antipyrin, carbolic acid, and naphthol, or from vegetable substances such as *Vicia faba*. The specific group comprises blackwater fever and paroxysmal haemoglobinuria. At first glance such a classification appears of assistance to those who are likely to come in contact clinically with blackwater fever cases, eliminating from the sphere of blackwater fever those confusing elements which are introduced if what is in reality a symptomatic or toxic haemoglobinuria is erroneously attributed to a specific disease.

Before accepting this classification, however, it may be well to consider in some detail the signs and symptoms by which these varieties of haemoglobinuria are said to be distinguished from one another. In Table I, compiled from these authors, are given in comparative columns the signs and symptoms under each form of haemoglobinuria, the symptomatic group being represented by malaria, the toxic by quinine, and the specific by blackwater fever.

Reviewing the table, it is worthy of note that the signs and symptoms of quinine haemoglobinuria resemble those of an attack of blackwater fever but are not so severe, and that jaundice is specially mentioned as being slight or absent in the former condition. The malaria group is allotted six positive signs and symptoms, which

TABLE I

Comparison of signs and symptoms of Tropical Haemoglobinurias.

Symptomatic	Toxic	Specific
Haemoglobinuria in malaria, Raynaud's Disease, Acute Specific Fevers and after severe Burns	Haemoglobinuria caused by Quinine, Chlorate of Potash, Antipyrin, Carbolic acid, Naphthol and *Vicia faba*	Haemoglobinuria in Blackwater Fever and Paroxysmal Haemoglobinuria
Malaria	**Quinine**	**Blackwater Fever.**
1. Haemoglobinuria 2. Fever 3. Rigor 4. Vomiting 5. Prostration 6. Anaemia Negative.—Rarity of severe Jaundice	Resemble those of an attack of Blackwater Fever, but are not so acute. Jaundice slight or absent.	1. Haemoglobinuria 2. Fever 3. Rigor 4. Vomiting 5. Intense weakness 6. Anaemia *Additional.* (*a*) Anorexia (*b*) Headache (*c*) Pains Back and Legs (*d*) Nausea (*e*) Diarrhoea (*f*) Thirst (*g*) Constipation (*h*) Jaundice (*i*) Hyperpyrexia (*j*) Coma

also occur in the quinine and blackwater fever columns. Additional signs and symptoms are enumerated under blackwater fever, and these evidently apply also to the quinine group, since the signs and symptoms of the latter are said to resemble those of an attack of blackwater fever but are not so severe. The value of these additional signs and symptoms as a means of distinguishing the blackwater and quinine groups on the one hand from the malaria group on the other, appears to be entirely discounted by the fact that these signs and symptoms are all, without exception, adduced by the authors in their foregoing description of one or other form of the malaria infections.

In the analysis of the differential diagnosis we find ourselves reduced to the following:—

(1) In the malaria group, the rarity of severe jaundice.

(2) In the quinine group, the relative lack of severity of the symptoms and the fact that jaundice is absent or slight.

Jaundice.

The patient whose chart is given below and who died of blackwater fever presented, some weeks before the date of the commencement of the chart, slight jaundice, which passed off in a day; he had a similar slight transient jaundice a week before his fatal attack of blackwater fever. At the time of the second attack of mild jaundice he had subtertian parasites in his blood in small numbers, and he had also taken quinine irregularly. To what, then, are we to attribute these mild attacks of jaundice? To haemolysis from malaria, or quinine or blackwater fever? It appears legitimate to assume that they were a manifestation of the same causes of haemolysis as produced the marked attack of blackwater fever. It is of importance to note that in this case during the fatal attack the jaundice was not of an intense kind. Deep jaundice, again, is known to occur in malaria; in fact, many authors include the 'yellow fever-like type' of malaria in their description. One of the cardinal signs of this type is deep jaundice.

From a study of this table of differential aids, one must conclude that although it may in the future be possible to distinguish accurately between a malaria, a quinine and a blackwater haemoglobinuria, this cannot by such aids be done to-day; the attempt at differential diagnoses on such slender evidence as the degree of jaundice and the severity of the symptoms is unscientific. It is commonly stated that blackwater fever is, owing to its severity, a condition which leaves no doubt in the mind as to the diagnosis. But if blackwater fever is a disease which presents itself in an acute form, and in an acute form only, then it is, indeed, a disease *sui generis* and incomparable with any other known disease.

Stephens' views on blackwater fever are quoted by the authors: 'Blackwater is not a disease *per se*, but rather a condition of blood in which quinine, other drugs, cold or even exertion, may produce a sudden destruction of red cells. The condition is produced only by malaria, and generally by repeated slight attacks, insufficiently combated by quinine. In such cases of chronic malaria, *i.e.*, in those suffering from anaemia, with repeated attacks of fever and repeated doses of quinine, blackwater fever sooner or later almost certainly supervenes, at least in tropical climates.' The authors' comment upon Stephens' account is as follows:—'These statements are too

sweeping if genuine blackwater is meant, otherwise the home of the disease would be Ceylon, whereas it is so rare that we have never heard of a genuine non-imported case; for in this island there are Europeans and natives with just the conditions required by Stephens, and yet they do not develop blackwater fever, because the only two cases which we have met with or heard of in Ceylon in twelve years were most probably cases of quinine haemoglobinuria. On the other hand, Stephens' remarks are correct if applied to quinine haemoglobinuria.' The last sentence of this criticism is important. If it be a fact that in Ceylon there are Europeans and natives with just the conditions required by Stephens, and if it be a fact that Stephens' remarks are correct if applied to quinine haemoglobinuria, how are we to explain the low prevalence of quinine haemoglobinuria in Ceylon, *i.e.*, two cases in twelve years?

The observation was made in this case of the occurrence of transient jaundice on two occasions before the severe attack of blackwater fever, malaria parasites being present on the second occasion; these preliminary attacks of jaundice may have represented the occurrence in the blood of—in a less degree—the same changes produced by the same cause as was active during the attack. The probability of such mild haemolytic attacks is great and they are easily overlooked by the patient, as they were in this case. It is also unlikely that anything short of a severe attack will attract the attention of the patient to his urine, and if the attention is not drawn to the urine, it is certain that under the conditions of life in such places as Africa a person will frequently fail to notice that his urine is abnormal. In order to observe even considerable degrees of haemoglobinuria, it is necessary to examine the urine in a suitable vessel in a good light, precautions not usually possible for patients living under the conditions which prevail in places where blackwater fever occurs. I would suggest, then, that closer investigation will reveal the fact that haemoglobinuria occurs frequently in the tropics without being observed, and that still more frequently haemolysis with slight jaundice occur without noticeable haemoglobinuria, and that these conditions are in fact frequently due to the same causes as blackwater fever and are mild forms of the same condition. Even in England, one has seen a case who was walking about and was unaware of the fact that he was passing haemoglobin in the urine in quite noticeable quantity.

Short of 'blackwater' fever, which represents a gross haemoglobinuria, there must be many degrees of haemolysis, haemoglobinaemia and slight haemoglobinuria produced by exactly the same agencies as produce 'blackwater.' For such cases I would suggest that the term 'blackwater' fever is not sufficiently comprehensive. We require a term for such conditions to indicate that the process of haemolysis has not produced such a degree of haemoglobinaemia as to result in the passage of haemoglobin in the urine.

Numerous suggestions as to the nature of the causal parasite of blackwater fever have been made during the last thirty years. Protozoa, bacteria, spirochaetes and chlamydozoa are represented among the suggested parasites. The suggestion of Sambon that blackwater fever might be due to a piroplasma-like parasite has been accepted by some, and there are many points of resemblance between this condition in man and piroplasmosis in animals. Dudgeon (1920) injected sterilized urine from cases of blackwater fever—obtained during the period of haemoglobinuria—into animals, without producing any ill-effects. This observer mentions as a possibility that the disease may be caused by a filter passer.

Experimental Inoculation of Blackwater Fever Blood

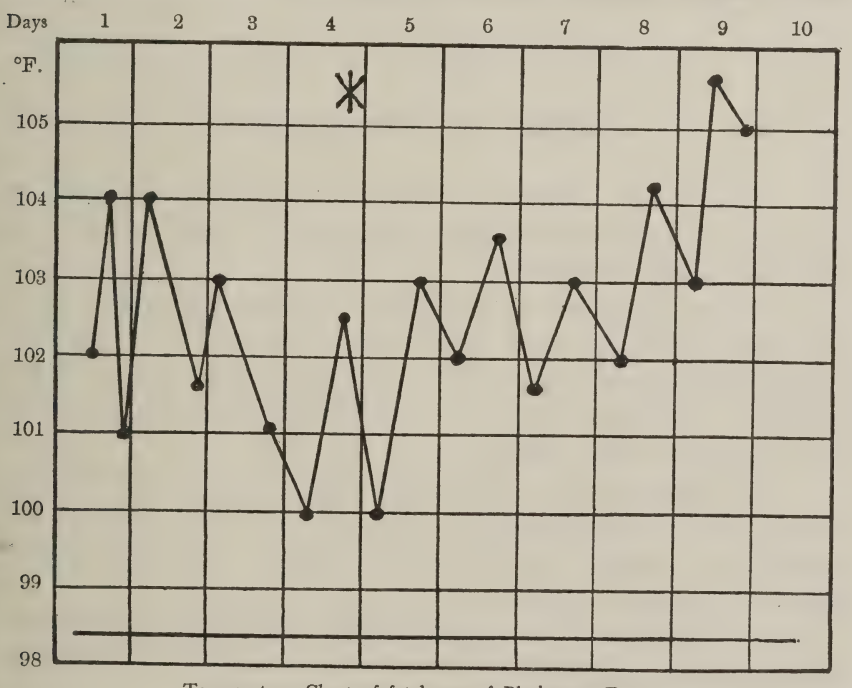
In order to throw some light upon this important question of whether or not there is a specific parasite or enzyme which causes blackwater fever, an experimental inoculation was performed. Blood was taken from the patient in the middle of what proved to be a fatal attack of blackwater fever, and was injected into a healthy European. The blood was withdrawn from a vein in the arm into a syringe containing citrated saline solution (2 per cent. Sod. cit. and 0·85 per cent. Sod. chloride, equal parts) and was injected in two portions into the recipient. The proportion of blood to citrate saline solution was three to one, and of this about 10 minims was injected deep into the region over the deltoid muscle at 3.45 p.m. and 2 c.c. into the same region at 4 p.m. The recipient had previously had malaria, the last infection being of the subtertian variety, but he had been free from relapse for over eighteen months and had taken no quinine for over eight months.

There was no local or general reaction immediately following the injections. Quinine bihydrochloride was administered orally in order to obviate infection with malaria. The dates, times and doses were as follows:—

December 4	...	10 grs.	...	4 p.m.
	...	5 grs.	...	8 p.m.
	...	5 grs.	...	10 p.m.
December 5	...	5 grs.	...	9 a.m.
	...	5 grs.	...	1 p.m.
		30 grs.		in 21 hours.

It might be argued that the doses of quinine taken might be capable of killing the parasite causing blackwater fever. Against this we have the record of numerous cases of blackwater fever in which even large doses of quinine failed to abate or ameliorate the condition. Also in the fatal case in question, quinine was administered by intramuscular injection on the sixth and seventh days of the disease, 21 grains in all, without influencing the temperature or improving the general condition.

Appended is the chart of the case giving the highest and lowest temperatures recorded each day. It will be observed that at the



Temperature Chart of fatal case of Blackwater Fever.

* Time at which injection was made.

time of injection the temperature of this patient was over 102° F., and that it remained high till his death five days later. It seems likely that if there was an infective agent it should have been present in the blood on the day of inoculation. Sources of fallacy include the possibility that the parasite of blackwater fever is never present in the blood at all; that it is present in such small numbers that the amount injected did not include the organism; that the parasite is present only for one or two days at the commencement of the disease; that it has an unusually long incubation period; or that the subject of inoculation was immune.

Result of inoculation

No immediate nor late effects were noted as the result of the inoculation. Parasites were not found in the blood, nor was there any rise of temperature nor haemoglobinuria observable during a period of two months.

These facts appear to me to militate against the specific parasitic theory of the etiology of blackwater fever.

SUMMARY AND CONCLUSIONS

1. The term 'Blackwater' Fever, being applicable only to conditions in which haemoglobin is present in visible quantity in the urine, is too restricted.

2. The importance of pre- and post-haemoglobinuria states which are inherent parts of the disease, is apt to be lost sight of owing to the exclusive use of the term 'Blackwater' Fever. Some such term as 'Occult' or 'Subliminal' Blackwater Fever might be used to express these conditions.

3. A differentiation of Tropical Haemoglobinurias into Malaria, Quinine and specific Blackwater types is not possible merely on the basis of the presence and degree of jaundice, or on the relative severity of the signs or symptoms.

4. The existence of a parasitic cause of Blackwater Fever has been frequently suggested; an experimental human inoculation, with

blood from a severe case of Blackwater Fever which ended fatally, elicited no evidence in favour of the existence of such a parasite after an observation period of two months.

ACKNOWLEDGMENT

I have to thank Dr. J. Y. Wood, W.A.M.S., for performing the inoculations.

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A NEW SPECIES AND A NEW VARIETY OF *CULEX* FROM THE BELGIAN CONGO

BY

A. M. EVANS

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The new species and variety of Culex described in the present paper occurred among two small collections of mosquitoes from the Belgian Congo, which were sent by Dr. Severin to this School for determination.

Culex moucheti, n. sp. (fig. 1).

This species is named in honour of its discoverer, Dr. Mouchet.

Head. Scales bordering the eyes white, flat below, and becoming gradually narrower as they approach the vertex. Upright forked scales golden yellow in front, very dark brown behind, intermediate ones golden with very narrow black tips. Narrow curved scales silvery white. Bristles projecting over vertex golden yellow. Scales of proboscis, female palpi, and scales and hairs of male palpi dark sepia.

Thorax. Prothoracic lobes with whitish scales. Mesonotum dark brown with bronzy scales, apparently discoloured by dampness, and blackish bristles. Pale whitish scales on ante-scutellar space, scutellum, and medially just behind the head. Pleurae with green integument. Lower mesepimeron of type with one bristle socket on left side.

Abdomen. Tergites II to VII entirely covered with dark sepia scales, except at side of tergites IV to VII, where basal triangular patches of whitish scales may occur; these very small, except on segment VII. Tergite VIII with large irregular patch of whitish scales above. Sternites entirely whitish scaled.

Legs. Vestiture chiefly blackish-brown. Femora pale beneath; middle tibiae and tarsi in some specimens with whitish scales beneath, extending for the whole part of the length of the segments.

Wings. Plume scales on third vein ligulate, squames on costa and first vein with six to eight striae. First fork cell slightly longer than its petiole, its base distal to that of second fork cell.

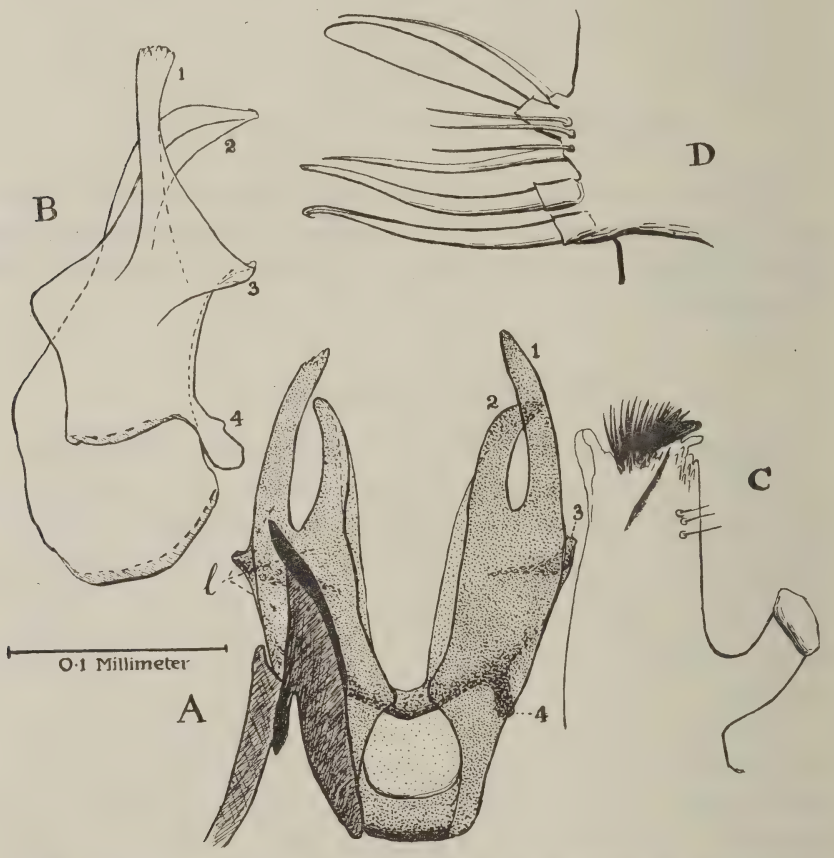

FIG. 1. *Culex moucheti*, n.sp. Male hypopygium. *A*—phallosome, ventral aspect with left parameral plate and part of basal plate; 1, 2, 3, and 4—processes of phallosome, *l*—left side. *B*—left half of phallosome, postero-lateral aspect somewhat distorted, 1, 2, 3, and 4, as in *A*. *C*—tenth sternite, ventral aspect. *D*—lobe of side-piece.

Male hypopygium (fig. 1). Lobe of side-piece as in fig. 1, *d*. The occurrence of only one moderately stout rod between the leaf and the pair of very stout rods a constant feature in the five specimens examined. *Phallosome* (Christophers, 1922) or mesosome

(Edwards, 1920) (fig. 1, *a* and *b*) of simple structure, chitin of walls thin and uniform except in region of basal tooth (4). Each half of phallosome with two long terminal processes (1 and 2), the longer weakly serrated distally, a short pointed process (3) projecting dorso-laterally, and a chitinised tooth (4) at basal angle of dorsal wall. Tenth sternites as in fig. 1, *c*.

Type ♂, six co-type ♂♂, and three co-type ♀♀ from Stanleyville, Belgian Congo, 1922, Dr. Mouchet.*

This species is one of the *pipiens* group of *Culex*, as defined by Edwards (1922). The hypopygial characters point to a relationship with *Culex pipiens*, L., and *C. trifilatus*, Edwards, but the absence of abdominal bands and of lines of white scales beneath the last two male palpal segments would seem to indicate affinities with the *decens* series of the group.

Culex annulioris var. *congolensis*, n. var.

Male resembling typical *C. annulioris*, Theo., in hypopygial characters, banding of palpi, proboscis and tarsi, and scaling of thorax, but with *abdomen entirely dark scaled above*, the median basal and lateral apical white markings characteristic of *annulioris* being entirely absent.

Type and co-type ♂♂ from Leopoldville, 1922, Dr. Duren.

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* Since going to press two further ♂♂ of *Culex moucheti* have been received from Buta, Belgian Congo, November, 1922, Dr. Mouchet.

REPORT ON SLEEPING SICKNESS IN EKET DISTRICT, SOUTHERN NIGERIA

BY

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(*Received for publication September*, 1922)

With the object of ascertaining the present position of sleeping sickness in the Eket District, Southern Nigeria, a tour of the whole district was made during April and May, 1922.

The route followed was from Oron to Awa, thus passing through the centre of the Eket District. The main towns at which I stopped and made enquiries were Oyubia, Ikorubo (site of old sleeping sickness camp), Eket and Awa, whilst numerous small, so-called villages were also inspected.

For the sake of clearness, and to avoid confusion, this report is divided into three parts:—

(1) Result of cases recorded in the year 1912.

(2) Result of cases not recorded in 1912, but who gave definite information that they had been inmates of the camp.

(3) General enquiry into trypanosomiasis in the Eket District during the months of April and May, 1922.

The whole investigation has been most difficult, the people showing the greatest reluctance to impart any information on the subject, and it was only after prolonged interviews, which were most wearying, that eventually the information detailed below was obtained. Very great assistance was given by the District Officer, as well as by members of the Qua-Ibo Mission.

PART I

In Macfie's Report (1915) on sleeping sickness in the Eket District in 1913, the following passage occurs:—

> 'During the sixteen months in which sleeping sickness has been under investigation, two hundred and twenty-two cases have been identified in which the trypanosomes have been demonstrated. In addition one hundred and fourteen cases have been met with presenting some of the clinical features of the disease, but in which parasites have not actually been found. There can be little doubt that the majority of these were cases of trypanosomiasis.'

The nominal roll of cases made by Macfie has unfortunately been lost, but I have assumed that the two hundred and twenty-two cases noted by him include eighty-nine definitely recorded cases, lists of which, either in manuscript or typescript, were compiled in 1912 by Foran and Gray, with a signed statement to the effect that all had been diagnosed microscopically.

Of these eighty-nine cases I was able to trace thirty-five, details of which are noted in Tables I and II. The remaining fifty-four cases have not been traced and nothing is known about them.

TABLE I.

Cases recorded in 1912 by Dr. Foran and Dr. Gray, and which are still alive.

Name	Age	Sex	Residence	When recorded	Remarks
Udo	9	M	Edem Idem Ekpot	8.8.12	Seen April, 1922. Healthy. No glands, but thickening on sides of neck which shows scars.
Usundurus ...	9	F	Ikorubo	7.8.12	Not seen. Is reported to be alive and well.
Enoesiet	adult	M	Afaha Eket	8.8.12	Seen 1922. Thickening on neck, with scars; no glands to be felt; strong and healthy.
Obotnt Ekim ...	12	F	Edem Idem Ekpot	8.8.12	Seen 1922. Strong and healthy; no glands to be felt.
Etok Awa ...	adult	F	Ikorubo	12.8.12	Seen 1922. Healthy; no glands to be felt. Slight scars on neck.
Adia	6	F	Idikpa	15.8.12	Seen 1922. Healthy; no glands to be felt. Slight scars on neck.

TABLE I—*continued*

Name	Age	Sex	Residence	When recorded	Remarks
Ekanem	11	M	Ikorubo	16.8.12	Not seen. Left the country apparently well.
Usoanwan ...	adult	F	Ikotesiokong	20.8.12	Seen 1922. Healthy. One small, very hard gland on neck.
Umwa Etok ...	7	F	Ikorubo	21.8.12	Seen 1922. Healthy; no glands or scars.
Amame	10	F	Ikotesiokong	24.8.12	Not seen. Alive and well, but refused examination.
Mama	17	F	Ikotesiokong	24.8.12	Not seen. Alive and well, but refused examination.
Okposen	adult	M	Ikotesiokong	27.8.12	Seen 1922. Healthy; scars on neck; three very small hard glands to be felt.
Adiah Ansudo ...	9	F	Ikotoquot	28.8.12	Not seen. Said to be alive and well.
Akpanitauwen ...	12	M	Ikorubo	28.8.12	Seen 1922. Healthy; one small gland on neck freely moveable.
Owoimaha ...	18	M	Afaha Eket	31.8.12	Seen 1922. Scars and thickening of neck.
Esoena	adult	M	Ikotesiokong	2.9.12	Seen 1912. No glands, but considerable thickening both sides of neck.
Peter Nsooyo ...	adult	M	Ekpenobo	11.9.12	Seen 1922. No glands, but scars both sides of neck. Strong and healthy.
Ntanwoo ...	14	F	Efrieyo	13.9.12	Seen 1922. Healthy; a few very small hard glands felt. Gland puncture refused.
Samuel Akpanuso	adult	M	Ikorubo	24.9.12	Seen 1922. Healthy; a few small glands to be felt; gland puncture negative.
Eya	17	F	Effoe	22.9.12	Seen 1922. Healthy; no glands; some thickening.
Ema	9	F	Akai	20.5.12	Seen 1922. Healthy; scars and thickening, but no glands to be felt.
Wilson Akpan ...	9	M	Ikorubo	?	Seen 1922. One small gland felt; gland puncture negative.
Adiaha Esein ...	13	F	Inoiya	28.5.12	Seen 1922. Healthy; some scars and thickening of neck.

TABLE II.

Cases recorded in 1912 by Dr. Foran and Dr. Gray, and which have died.

Name	Age	Sex	Residence	When recorded	Date of Death	Alleged cause of Death
Adiansun... ...	18	F	Ikorubo	15.8.12	1918	Influenza.
John Opan ...	adult	M	Ikorubo	17.8.12	1921	Small pox.
Adong	9	F	Ikotesiokong	17.8.12	1916	Sleeping sickness.
Samso Okpa ...	12	M	Ikotesiokong	20.8.12	1921	Small pox.
Ikotumoanwan ...	9	M	Ikotoquot	22.8.12	1915	Sleeping sickness.
Edikpoi	18	M	Ikorubo	27.8.12	1912	Sleeping sickness.
Udouqua... ...	13	M	Ikotesiokong	27.8.12	1912	Sleeping sickness.
Eno	8	F	Okong	27.8.12	1918	Influenza.
Ekpo Awan ...	8	M	Ikotodiong	2.9.12	1918	Influenza.
Esukoku	12	M	Ikotesiokong	7.9.12	1915	Sleeping sickness.
Ikpeisak	11	F	Ikotesiokong	20.9.12	1915	Sleeping sickness.
Idimedoho ...	15	M	Ikotekong	23.9.12	1918	Influenza.

In the twenty-three cases still alive, noted in Table I, the blood was examined by wet and dry films, but in no case were trypanosomes found. It must be noted, however, that Gallagher and Macfie failed to discover trypanosomes in the blood of any of the cases, diagnosis in every instance being made by gland puncture.

Very few glands were punctured during the present enquiry, as in practically all cases the glands had resolved, or there was at most an indefinite general thickening. In a few cases there were one or two very small, extremely hard glands to be felt, fibrosis evidently having taken place. Scarring of the neck to a variable degree was common.

PART II

Apart from these thirty-five *recorded* cases, I saw at Eket twenty-eight *unrecorded* cases whom I accepted as being former patients at the camp from their history and from evidence, especially from members of the Qua-Ibo Mission, who furnished me with

documentary proof of their personal knowledge of the individuals. These cases were all in good condition, and did not exhibit enlarged lymphatic glands or any other evidence of trypanosomiasis.

I was also informed of the deaths of seven *unrecorded* cases who were stated to have been inmates of the camp.

It is possible that the above-mentioned unrecorded cases are included in Macfie's one hundred and fourteen cases who presented some of the clinical features of the disease, but in whom parasites were not actually found.

PART III

In endeavouring to ascertain if trypanosomiasis is still prevalent in the Eket District, as many persons as possible were examined in the towns and villages visited. As already stated, great difficulty was experienced, as there appeared to be a very great reluctance to impart information; in fact, so much so, that on several occasions it was necessary to invoke the assistance of the authorities in order to get the people of a particular place to come in for examination. At no place was this more marked than at Ikorubo, the site of the former sleeping sickness camp. I am informed that this was due to the fact that the chiefs and headmen of the various surrounding villages feared being called upon to furnish labour for another and new sleeping sickness camp.

Twenty-three cases were seen which exhibited signs suggestive of sleeping sickness, *e.g.*, enlarged glands, but gland puncture and blood examination were negative.

In all, one thousand eight hundred and six persons have been examined by gland palpation, and the following table shows the result. For purposes of comparison with the results recorded by Macfie and Gallagher (1914), the same age-groups classification of glands has been adhered to:—

+	= Glands obviously enlarged.
+ −	= Sufficiently enlarged to be grasped.
+ − −	= Enlarged, but not sufficiently to be grasped.
−	= Normal.

TABLE III.

The incidence of enlarged posterior cervical glands among 1806 natives classified according to sex and age.

Sex	Male			Female		
Age	0-13	14-44	45-	0-11	12-39	40-
+	5 (0·88%)	4 (0·64%)	0	2 (0·64%)	4 (1·42%)	0
+−	40 (7·04%)	7 (1·13%)	1 (5·0%)	22 (7·1%)	13 (4·63%)	0
+−−	372 (65·5%)	210 (33·87%)	3 (15·0%)	171 (55·16%)	62 (22·06%)	0
−	151 (26·58%)	399 (64·35%)	16 (80·0%)	115 (37·1%)	202 (71·88%)	7 (100·0%)
Number of individuals of each class examined ...	568	620	20	310	281	7

As regards the above table, it will be seen that a large proportion have glands that can be classified as + − − ; those with + − are considerably smaller, whilst the + is a very small figure to the total. The class −, or normal, equals 49·28 per cent. of the total examined. In plain words, it is very rare to see anyone who has enlarged glands to an extent that is noticeable. Enlarged glands are certainly common, and they usually take the form of small discrete, hard, shotty glands; in many cases they can be very well compared with buckshot. In a few cases the glands were observed to be suppurating, with a well marked sinus, or they were distinctly soft; now and again the post-auricular ones were enlarged, but this was comparatively rare.

Comparison of the above tables with those of Macfie and Gallagher shows a striking reduction in the proportion of individuals with enlarged glands.

The number of 'dirty heads' was most marked, ranging from a simple dry eczema to the most intensely deep punched-out necrotic ulceration. There is no doubt that the 'dirty head' was more common amongst males, and in a large majority of the heads classified 'dirty' the occipital glands were always involved.

Many hundreds of blood slides have been examined, both by the wet and dry methods, but in no case was a trypanosome found; similarly, gland punctures performed whenever possible were invariably negative.

The people of Eket are of a very poor physique, and many of them have the appearance of being ill-nourished and on the verge of starvation. Various diseases—yaws, syphilis, rheumatism—appear to be common. Eket is densely populated, the inhabitants living in scattered houses, and not in any definite towns.

Very few biting flies were found, but a few tsetse were captured.

SUMMARY

Of the cases seen and referred to in the Annual Medical Report for the year 1913, thirty-five cases have been traced out of a total of eighty-nine recorded by Foran and Gray, and of these twenty-three are alive and in good health (Table I).

In addition, twenty-eight cases have been traced who gave direct information as to their having been in the camp, but whose names were not recorded or cannot be traced. It is possible, however, that some of these twenty-eight cases were amongst the one hundred and fourteen noted by Macfie as presenting some clinical signs of the disease but in whom the condition was not diagnosed microscopically. In this connection it is gratifying to be able to state that the names of the various medical officers who had charge of the camp are well remembered, and on more than one occasion the name of the doctor in charge was given without seeking.

Sleeping sickness has not been demonstrated during the recent tour in Eket, although the cases selected for examination were chosen as presenting clinical signs of possible trypanosome infection. The natives themselves are of the opinion that the disease has died out, and this statement is borne out by the members of the Qua-Ibo Mission; at the same time this must not be accepted as a definite statement, because, as already noted above, the natives are not inclined to discuss the subject, and I am strongly of the opinion that a number of possibly genuine cases were removed from villages on my approach.

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NOTES ON CULICIDAE IN VENEZUELA, WITH DESCRIPTIONS OF NEW SPECIES

PART III

BY

A. M. EVANS

(*Received for publication 27 February*, 1923)

Anopheles (*Arribalzagia*) *punctimacula*, D. and K.

Anopheles (*Arribalzagia*) *venezuelae*, Evans

Amongst the *Arribalzagia* material from the Panama Canal Zone, referred to in my previous paper (1922), were twenty-nine specimens with at least one of the hind tarsi complete. Most of these agreed with Howard, Dyer and Knab's (1917) description of *A. punctimacula*, D. and K., but two specimens had two hind tarsal bands as in *A. venezuelae*, and two others showed a tendency to the formation of the second band. Further, a very considerable amount of variation was found among the specimens, with regard to the spotting of the other segments of the tarsi, the left and right hind legs of the same insect in one case being markedly different in this respect. The tarsal characters used by me to distinguish *A. venezuelae* from *A. punctimacula* (1922, p. 217) are, therefore, valueless.

I have also been able to examine numerous other examples of *A. venezuelae*, kindly sent by Dr. Núñez Tovar from Venezuela, among them being seven specimens in which the last hind tarsal segment has only one dark band. It was also found that the most perfect specimens among these collections had a number, from two to fourteen, dark squames scattered throughout the long pale scaled area of the third vein, thus agreeing with the description of *A. punctimacula*, D. and K.

A re-examination of the type of *A. venezuelae* has revealed the fact that one of the wings has several dark scales in this position. I have, therefore, no hesitation in regarding *A. venezuelae*, Evans, as synonymous with *A. punctimacula*, D. and K.

Culex maracayensis, n. sp. (fig. 1)

MALE.

Proboscis with a narrow whitish band on outer third. *Palpi* with scales mostly dark brown, pale scales creamy, forming a narrow band on basal half and a wide band on proximal half of long segment, bases and apices of all the segments pale scaled. *Occiput* with silvery narrow curved scales in front, brassy ones behind, upright forked scales blackish. *Prothoracic lobes* with whitish narrow curved scales and pale brown bristles. Integument of mesonotum reddish brown. Dorsum with two broad bare stripes, narrowing distally. Vestiture of rather sparsely distributed golden brown and silvery scales, the latter occurring chiefly at anterior lateral margins, on anterior fourth of median area, around anti-scutellar space, and in two small oval areas on posterior half of disc. Bristles, numerous, brown.

Abdomen. Tergites dark brown scaled with narrow irregular basal bands of whitish scales. Sternites clothed with transparent whitish scales.

Wings with dark brown scales. Bases of fork cells about equidistant from base of wing. First fork cell about twice as long as its petiole.

Legs. Femora pale beneath, the pale area being very well defined on the femur. Apices of front and mid femora narrowly pale. Front tibia with conspicuous apical white patch above, about twice as long as the average width of the tibia in dorsal aspect. Mid tibia with very small pale apical spot, pale scaled beneath throughout, hind tibia with a well defined stripe of creamy scales extending along most of its length dorsally and a well defined apical white ring. Front and mid tarsi with first two segments narrowly pale apically, other segments of front tarsi without white, those of mid tarsi with one or two pale scales apically. Hind tarsus, with conspicuous pale rings apically and basally on all the segments.

Hypopygium (fig. 1). Side-pieces (A) with clasp narrowing gradually towards distal extremity, articulated spine narrow. Lobe of side-pieces (B) an undivided, distally directed arm, bearing three stout rods, of which two are sub-equal, and longer and stouter than

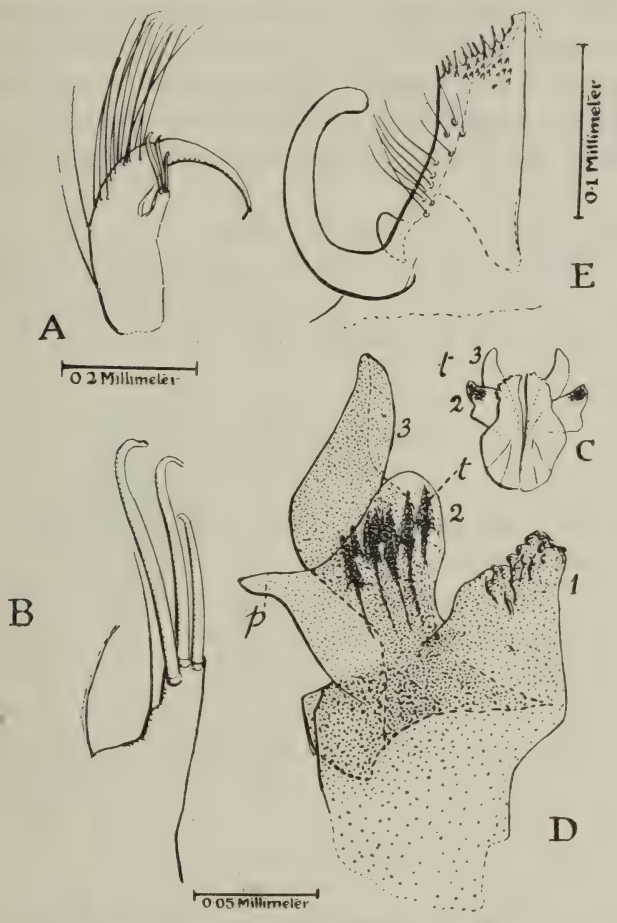


FIG. 1. *Culex maracayensis*, n.sp. Hypopygium. *A.*—Side piece. *B.*—Lobe of side piece. *C.*—Phallosome, ventral aspect, semi-diagramatic, to same scale as *A*; 1, 2, 3 lobes numbered as in *D.*; *t.*—position of teeth. *D.*—Half of phallosome under pressure, ventro-lateral aspect; *p.*—process of lobe 2; *t.*—teeth on upper side of lobe 2. *E.*—Tenth segment, dorsal aspect.

the third. *Phallosome* (C & D) with each half divided distally into three lobes; inner lobe (1) ventral, with chitin distally thrown into ridges, which give rise to a denticulate appearance, particularly at the margin. Second lobe when flattened out appearing as a thin

plate with an external pointed process (p.) and bearing on its dorsal surface a row of blackish chitinous teeth, five large and three or more small (some of these teeth are largely obscured by the others in the figure); four of the large teeth continued proximally as thin chitinous ribs. Third lobe (3) arising dorsally to second lobe, elongate, curved and much narrower than the first and second lobes. Tenth segment a membraneous lobe with curved basal arms, distal margin spinose, tergal surface with paired chitinous plates, a spinous area distally, and a group of four setae and a row of seven setae laterally.

Length, c. 4·0 mm. Wing, c. 3·0 mm.

Type: One ♂, Maracay, October, 1922; Dr. Núñez Tovar.

This species appears to be most closely related to *C. coronator*, which it resembles in colouration and in the character of the tenth sternites.

Culex paganus, n. sp.

MALE.

Palpi very short, as short as those of female. *Head:* Antennae plumose, hairs brown; *proboscis* dark brown scaled, expanded apically; *eyes* black, *occiput* black clothed with white, narrow curved scales, white flat ones at sides below, and pale yellowish brown upright forked scales. Clypeus yellowish brown, sub-globular.

Prothoracic lobes whitish scaled. Integument of mesonotum pale olivaceous, darker where sub-median bare stripes occur and in posterior lateral areas. Scales whitish and pale yellowish brown, the whitish ones predominating anteriorly and at sides. Bristles long, dark brown. Pleurae pale green.

Abdomen with grey integument. Scales of tergites dorsally very dark brown with sub-metallic bluish lustre, ventrally whitish with bluish lustre. Sternites whitish scaled.

Legs unbanded, vestiture dark sepia, femora pale beneath.

Wing. Scales of costa and sub-costa dark sepia, on other veins semi-transparent with obscure bluish tinge in certain lights. First fork cell almost three times as long as its petiole, second twice as long as its petiole.

Hypopygium. The main features are illustrated in figure 2. Tenth sternites slender, comb-shaped distally with about six teeth.

FEMALE.

Antennae pilose, hairs brown. Occiput with creamy narrow curved scales and pale straw-coloured upright forked ones. Mesonotum with integument uniformly brown, pale scales almost confined to edges of disc and lateral depressed areas.

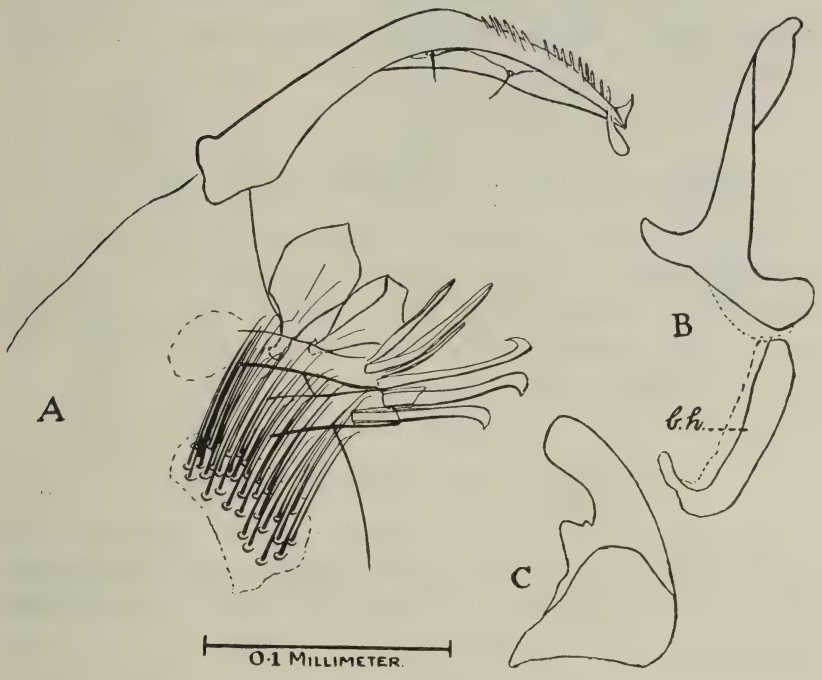


FIG. 2. *Culex paganus*, n.sp. Hypopygium. *A.*—Apical part of side piece, general setae of vestiture omitted. *B.*—Half of phallosome, lateral aspect; *b.b.*—basal hooks. *C.*—Transparent triangular plate.

Legs. Hind tibia and metatarsus with a few brassy scales beneath.

Type: ♂ and ♀ from villages, Estada Aragua, Venezuela, 23rd August, 1922; Dr. M. Núñez Tovar.

This species apparently approaches near to *Culex* (*Isotomyia*) *bifoliata*, Dyar, from the Panama Canal Zone, in the structure of the male hypopygium. The leaves on the stem of the upper division of the side-piece are, however, described as 'crooked curved leaves,' and although the leaves (*l.*) in *C. paganus* are apt to be folded in mounting, they could not appear crooked unless greatly distorted in this process. There are also a number of other differences in

points of detail, but in the absence of a figure of the structures in *C. bifoliata* it is difficult to estimate the value of these. In vestiture, however, *C. paganus* differs very greatly from the Panama Canal species, in which the upright forked scales of the head are white, the vestiture of the mesonotum consists of 'fine dark brown hairs, and the abdomen is entirely black.' There can be no doubt, therefore, that *C. paganus* is specifically distinct from *C.* (*Isotomyia*) *bifoliata*, Dyar.

Culex (*Neomelanoconion*) *chrysothorax*, Newst. and Thomas

I am now able to confirm the occurrence of this species in Venezuela, which has hitherto rested on the record of a single female collected by Professor Stephens at Mene Grande. Two males and two more females were taken at Maracay, 5th October, 1922, by Dr. M. Núñez Tovar.

Psorophora tovari, Evans (figs. 3 and 4)

A considerable amount of material of this species has been received since the publication of its description (1922), which enables me to give a comprehensive account of the thoracic and abdominal colouration, as well as a description of the male.

FEMALE.

Mesonotum. The distribution of scales of different shapes and colours is illustrated in figure 3. The narrow curved, spindle-shaped, and smaller broad curved scales (fig. 3, C, D & E), which are usually dull brown or yellowish brown, are in some specimens dull pale yellow and whitish. The very broad, much curved scales (B, B1) are usually pale creamy yellow, sometimes pale yellow.

Abdomen. The broad, pale yellow, apical, dorsal bands which are complete on segments two to six of the type, may be interrupted medially by dark scales on segments three to six, four to six, five to six, or six; or they may be separated from the posterior margins medially by a relatively small or large dark scaled triangular patch on these segments.

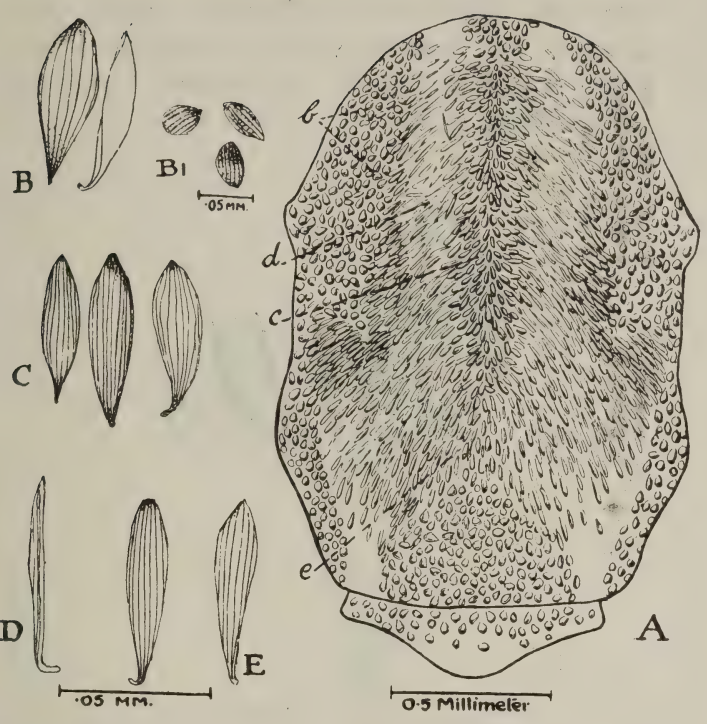


FIG. 3. *Psorophora tovari*, Evans. *A*.—Mesonotum of female. *B*., *C*., *D*., and *E*.—Scales from regions *b*., *c*., *d*., and *e*., of mesonotum, mounted in balsaam; *B* 1.—scales from region *b*. as seen in *situ*.

MALE

Palpi entirely clothed with blackish scales with deep metallic blue reflections and black hairs, last two segments incrassate. Hairs of antennae brown, tori shining black. Occiput, mesonotum, legs and wings as in female.

Abdomen with apical bands usually complete on segment two, complete or divided on segment three, and generally interrupted medially either partially or completely on the other segments.

Hypopygium (fig. 4). Claspettes (harpagones) with nine (this number may be subject to slight variation) stout filaments (*f*.) arising from prominences along distal border, and a row of about sixteen to twenty very delicate setae with distal portions swollen and produced into fine filamentous processes as shown in the figure.

Type: ♂ and nine co-type ♂♂ from Maracay Region, Venezuela, 1922; Dr. M. Núñez Tovar. Co-type ♀ from Maracay,

10th October, 1922, and others from Maracay Region, July, 1922, ♀♀ 14; Maracay, 5th June, 1922, ♀♀ 30; San Meteo, 2nd June, 1922, ♀♀ 27; Guacara, 1922, ♀♀ 4; Lagua, 15th June, 1922, ♀♀ 2. Dr. M. Núñez Tovar.

Fig. 4. *Psorophora tovari*, Evans. Apical portion of claspette, ventral aspect; *f.*—stout filament; *b.*—expanded hair.

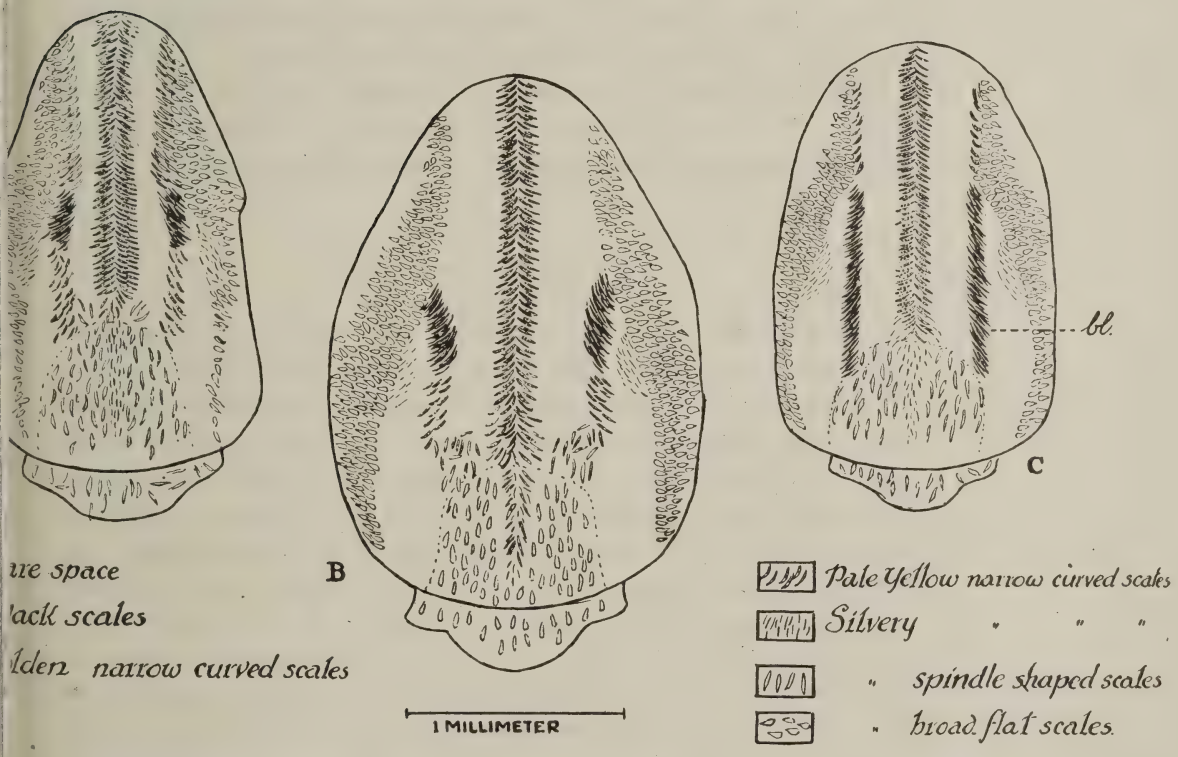
This species is evidently closely allied to *P. cyanescens*, Coq., and *P. purpurascens*, Eds., specimens occurring which resemble one or other of these species in abdominal markings. The three species appear to differ chiefly in mesonotal vestiture; *P. cyanescens* having 'broad soiled silvery scales intermixed with some narrower brown ones . . . . especially on centre of disc, *but not forming any defined pattern*' (H., D. and K., 1915), while *P. purpurascens*, Eds., has the mesonotum with 'flat silvery grey scales, darker, but not conspicuously so, in the centre of the mesonotum.'

Psorophora ciliata, Fab.

In a previous paper (1922) I recorded the occurrence of two specimens of this species near Maracay, and Dr. Núñez Tovar has subsequently sent further material from this region. In view of

Dyar's recent study of the species of the *ciliata* group of *Psorophora*, and their distribution, and also of the fact that they exhibit considerable differences in thoracic pattern from *P. ciliata*, a further discussion of the Venezuelan specimens is necessary.

Dyar recognises four species of the *ciliata* group of *Psorophora* in the Argentine region, and states that, apart from Theobald's record of it in British Honduras, true *ciliata* has not been recorded south of Tampico, Mexico. Further, he separated *P. tibialis*, a South American species, from *ciliata* by the slight differences of mesonotal pattern together with the markedly discontinuous distribution. Now, in none of the Venezuelan specimens does the mesonotal pattern conform exactly to that of *P. ciliata*, and in some cases (fig. 5, A and B) it differs quite as much as that of *P. tibialis*,



5. *Psorophora ciliata* from Venezuela. Mesonotal patterns of three different specimens. *A.* and *B.*—Female; *C.*—Male.

resembling this latter species in the reduction of the long lines of black scales (fig. 5, C, *bl.*). All the five specimens differ from *P. ciliata* in having the median line of narrow curved scales not golden in the antescutellar space. In three of the specimens these scales are entirely silvery (fig. 5, A & C), while in the other two (fig. 5, B) they are mostly pale yellow. Owing to the amount of variation which exists among only five specimens from this region, and the proximity of Venezuela to Central America, I regard these specimens as specifically identical with *P. ciliata*.

Megarhinus trinidadensis, D. and K.

Males, larvae and pupae agreeing with the description of this species, and females differing in the absence or reduction of white on the third mid-tarsal segment, have been received from Dr. M. Núñez Tovar. This difference does not seem to justify the separation of these specimens from the Trinidad species.

Bred in laboratory, Maracay, 1st November, 1922, Dr. M. Núñez Tovar, ♂ 1, ♀ 1; Mariara, Est. Aragua, 11th September, 1922, ♂ 1; Maracay, 4th June, 1922, ♂ 1; Maracay region, ♂ 1, ♀♀ 20.

Goeldia longipes, Fab.

Five females taken at Tucupido, December, 1922; Furmero, 8th June, 1922, ♀♀ 2; and Maracay region, June and July, 1922, ♀♀ 2, by Dr. M. Núñez Tovar, are referred to this species, although they differ slightly from Howard, Dyar and Knab's (1915) account of it. The mesonotal scales have a distinct sub-metallic blue colour, when the thorax is viewed from behind, and the scales on the scutellum and ante-scutellar space are peacock-blue and greenish-blue. In these respects they resemble *L. culicivora*, D. and K., but they differ from this species, and resemble *G. longipes*, Fab., in the ciliation and colouration of the hind legs. The female palpi are said to be equal in length to six antennal segments in *G. longipes*, and to four in *G. culicivora*; in the Venezuelan specimens the female palpi equal nearly five antennal segments, that portion projecting beyond the clypeus being equal to four segments.

Wyeomyia (Decamia) pseudopecten, D. and K.

A male specimen taken at Maracay, 2nd September, 1922, by Dr. M. Núñez Tovar, was found to agree closely with this species in hypopygial characters, but the long paired hairs of the side-piece, though longer than the clasper were less than twice its length.

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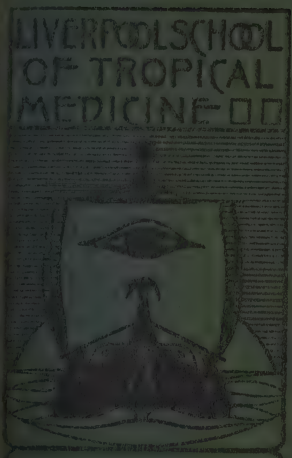

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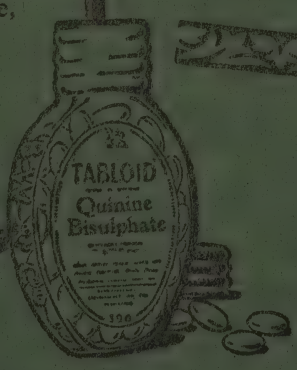

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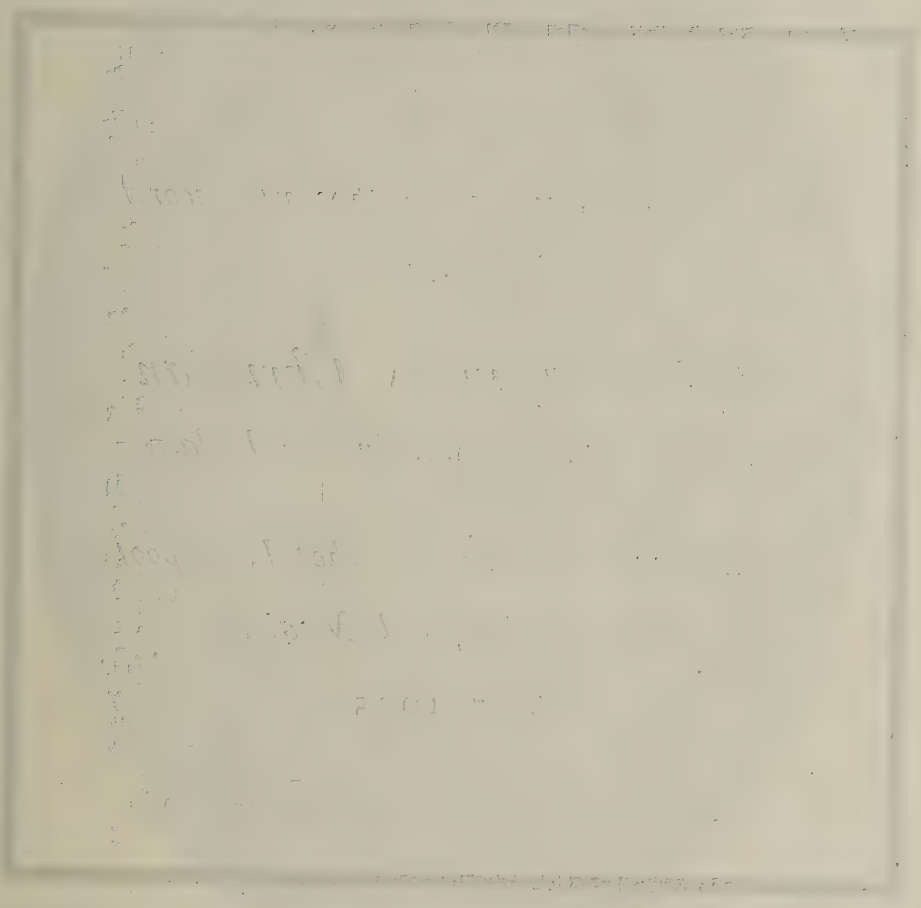

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>
> Smith, J. (1900). 'Enlargement of the spleen in malaria.' *Journal o Pathometry*, Vol. I, pp. 1-20.

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A REVISION OF THE *AMPHISTOMATA* OF MAMMALS

BY

P. A. MAPLESTONE

(*Received for publication* 12 *January*, 1922)

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* Species in the Museum, Liverpool School of Tropical Medicine.

INTRODUCTION

The publication by Stiles and Goldberger (1910) of their monograph on the 'PARAMPHISTOMOIDEA' called forth hostile criticism from many writers, notably Braun (1911), Odhner (1911), and Looss (1912). The chief objection of these authors to Stiles and Goldberger's classification is that they ignored the recent attempts that had been made to classify the Trematodes by using affinities in the lymphatic and excretory systems as a basis. Although not agreeing with the classification of Stiles and Goldberger, none of the above writers formulated an alternative system, and although Looss in his article of 1912 says he has been several years trying to group the Amphistomes on the comparative features of the lymphatic, excretory and copulatory systems, this work has not been published yet, so far as the writer is aware. The result is that the confusion caused by Stiles and Goldberger's multiplication of genera, etc., still exists. Stunkard (1917), in a resumé of the group, makes a 'provisional' attempt at reclassification, but as he did not examine any material except three or four species from American fish, for which he makes two new genera, he is unable to discuss whether many of the existing species and genera are valid or not. All that he has done is to remove Stiles and Goldberger's family names, leaving some of their sub-family and their generic names; to add a sub-family of Looss containing one of his own genera, one of Looss', one '(*Gen. nov.*) *spinulosum,*' and 'Genera of uncertain position'; and to recommend a new sub-family for *Balanorchis* and another sub-family for his own genus *Zygocotyle.* Such a procedure cannot be regarded as improving matters.

It is probable that Looss' suggestion to classify the members of the group on the minute anatomy of the lymphatic, excretory, and copulatory systems is sound, but it appears to be too complicated for practical purposes.

In view of the opportunity presented by the large collection of Amphistomes in the Museum of the Liverpool School of Tropical Medicine, the writer decided to undertake a revision of the group with the object of providing a working classification. This is not based on minute histological study but on easily ascertained anatomical characters, and some of the divisions used by Stiles and Goldberger (e.g., the three families *Gastrodiscidae, Paramphistomidae,* and *Gastrothylacidae*) have been retained because they serve to divide the group on easily distinguished external

characters, and are hence of practical use. It is realised that the present attempt at classification does not take cognizance of the standards required by the more advanced systematists such as Looss and Odhner, but it is claimed for this system that it is reasonably simple and consistent, and, so far as the Amphistomes of mammals are concerned, enables a species to be determined with considerable accuracy.

Looss also draws attention to the fact that Stiles and Goldberger failed to make any allowance for variation due to difference in age or the state of contraction of specimens, and have made many species on unsound data in consequence. Throughout the present work these factors have been investigated as fully as possible, and an attempt has been made to show to what extent they influence the appearance of the several species of the group. In their monographs Fischoeder and Stiles and Goldberger both deal so fully with the synonymy, that it has not been considered worth while to go back beyond these authors.

Note.—The reader is referred to Cohn (1904), Daday (1907), Looss (1912), and Stunkard (1917), for the most recent information regarding Amphistomes parasitic in hosts other than mammals.

AMPHISTOMATA, Rudolphi, 1801, e.p., Nitsch, 1819

Definition.—Digenea : two suckers, the anterior surrounding the mouth and the posterior terminal or ventro-terminal behind the genitalia ; gut forked ; excretory pore opening dorsally towards the hinder end ; testes generally in front of ovary ; almost always thick worms more or less circular in section.

Key to Families

1. Body usually flattened and divided into anterior and posterior portions ; ventral pouch absent ... ... *Gastrodiscidae*
   Body usually conical and not divided into anterior and posterior portions 2
2. Ventral pouch absent ... ... *Paramphistomidae*
   Ventral pouch present ... ... *Gastrothylacidae*

Family *PARAMPHISTOMIDAE*, Fischoeder, 1901.

Definition.—Amphistomata : body not divided into two portions ; ventral pouch absent.

Key to Sub-families

1.	Oral diverticula absent	*Paramphistominae*
	Oral diverticula present	2
2.	Oral diverticula double	*Cladorchinae**
	Oral diverticula single	*Stephanopharynginae*

Sub-family *PARAMPHISTOMINAE* (Fischoeder, 1901), s.str., Stiles and Goldberger, 1910.

Definition.—Paramphistomidae, without oral diverticula.

Key to Genera

Genital sucker absent	*Paramphistomum*
Genital sucker present	*Cotylophoron*

Genus *Paramphistomum* (Fischoeder, 1901), s.str., Stiles and Goldberger, 1910.

Definition.—Paramphistominae, without a genital sucker.

Type species *Paramphistomum cervi* (Zeder, 1790), Fischoeder, 1901.

Key to Species

A.	Testes in tandem†	1
B.	Testes diagonal†	6
1.	Testes lobed	2
	Testes not lobed	5
2.	Testes with two lobes	*P. gigantocotyle*
	Testes with more than two lobes	3
3.	Anterior sucker deeply retracted	*P. pisum*
	Anterior sucker not retracted	4
4.	Laurer's canal opens posterior to excretory pore	*P. cervi*
	Laurer's canal opens anterior to excretory pore	*P. orthocoelium*
5.	Laurer's canal opens posterior to excretory pore	*P. liorchis*
	Laurer's canal opens anterior to excretory pore	*P. wagandi*
6.	Testes lobed, Laurer's canal posterior to excretory pore ...	*P. explanatum*
	Testes not lobed, Laurer's canal anterior to excretory pore ...	*P. buxifrons*

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* Cohn (1904) created a new sub-family *Diplodiscinae*, all the members of which are found in Amphibians and Reptiles. This sub-family has two oral diverticula and has no constant characters by which it may be distinguished from *Cladorchinae*.

† Refers to mature worms, because in young specimens of *P. cervi*, the testes are sometimes found to be slightly diagonally arranged; by this it is meant that the testes are only slightly out of the mid-line, so that the antero-posterior axis of the worm always intersects both testes, but one testis always lies so far in front of the other that a transverse line can be drawn between them without touching either. In young specimens of *P. explanatum*, the testes lie one on each side of the mid-line, so that the antero-posterior axis passes between them without touching either, but one testis is only slightly in front of the other, so that a transverse line intersects both testes. In mature *P. cervi* the slightly diagonal arrangement is not seen, and the testes appear in tandem, whereas in mature *P. explanatum* the testes are definitely diagonal.

Paramphistomum cervi (Zeder, 1790), Fischoeder, 1901.

Synonymy :—

Paramphistomum gracile, Fischoeder, 1901.
Paramphistomum bothriophoron (Braun, 1892), Fischoeder, 1901.
Paramphistomum microbothrium, Fischoeder, 1901.
Paramphistomum bathycotyle, Fischoeder, 1901.
Paramphistomum epiclitum, Fischoeder, 1904.
Paramphistomum papillosum, Stiles and Goldberger, 1910.
Paramphistomum papilligerum, Stiles and Goldberger, 1910.
Paramphistomum indicum, Stiles and Goldberger, 1910.

The following is a list of the material available :—

1. Ten bottles from the stomach of bullocks killed in the military slaughter-house at Sierra Leone, West Africa.
2. Two bottles from the stomach of bullocks killed at Khartoum, Sudan.
3. One bottle from the stomach of a bullock killed at Yola, Northern Nigeria.
4. One bottle from the stomach of a bullock killed at Zomba, Nyasaland.
5. One bottle from the stomach of a bullock killed at Blantyre, Nyasaland.
6. One bottle from the stomach of a bullock killed at Accra, Gold Coast.
7. One bottle from the stomach of a bullock killed at Nairobi, Kenya Colony.
8. One bottle from the stomach of an antelope (*Cobus* sp.) shot in the Northern Territory of the Gold Coast.
9. One bottle from the stomach of a Roan shot in Nyasaland.
10. One bottle from the stomach of a bullock in England.
11. Two bottles, host and locality ?

Many of the above collections comprised some hundreds of specimens, and other worms of the same group occurred along with them in several instances.

External Anatomy

Size and shape. The size and shape exhibit such infinite variation as to be of little diagnostic value (Plate V).

Cuticular papillae. Fischoeder (1903) states that in some cases he found a number of small papillae of varying size on the cuticle surrounding the oral opening of *P. cervi*, and he does not agree with von Blumberg (1871) that these papillae are always present. The present investigation is in accordance with Fischoeder's view, as they were found in some specimens and not in others.

Other points usually discussed under External Anatomy will be included under Internal Anatomy, because their full consideration involves details that can only be made out in sections or in cleared specimens.

INTERNAL ANATOMY

Muscular system. The points requiring special mention in this system will be referred to under special organs.

Nervous system. This system was not investigated as it has not been used in specific diagnosis.

Excretory system. Fischoeder (1903) describes and figures the excretory bladder of *P. cervi* as being of a definite shape, with thin smooth walls and with the excretory pore opening well in front of the bladder. Examination of a large number of sectioned specimens leads one to the conclusion that a definite form cannot be assigned to the excretory bladder, which varies according to its degree of distension. The position of the excretory pore in relation to the bladder is also found to vary with the age of the individual; in those with no eggs in the uterus, the pore opens dorsally or dorso-posteriorly of the bladder, and as the uterus gradually fills with eggs the pore appears to pass further and further forward until it is seen to open well in front of the bladder. This point is fully discussed under *P. explanatum*, and as *P. cervi* is found to vary in exactly the same manner no further reference to it will be made here.

Anterior sucker. The anterior sucker may be longer than broad, circular, or even broader than long. When contracted the central canal of the sucker may be narrow; on the other hand if its external aperture is widely open, the canal may be funnel-shaped, becoming narrower towards its junction with the oesophagus. The internal surface of the oral cavity may, or may not, be furnished with small papillae, but these vary in exactly the same way as in the case of the cuticular papillae; in fact, owing to their irregular distribution, papillae may be present in some sections and absent in others of the same worm. Fischoeder (1903) states that he found papillae in all the sectioned specimens he examined.

Oesophagus. No special mention of the muscle wall of the oesophagus of *P. cervi* is made by Fischoeder (1903), but in describing other species, e.g., *P. dicranocoelium* and *P. cotylophorum*, he says that the oesophagus of these two worms is characteristic, because the muscle wall is thicker near the gut fork than near its anterior end; therefore the inference is that the oesophagus of *P. cervi* does not exhibit this thickening. Careful examination of five specimens of *P. cervi* cut in sagittal section gave the following results.

Table I.

Thickness of the muscle wall of the oesophagus of *P. cervi*.

	Near its anterior end	Near the gut fork
Specimen 1	32μ	60μ
Specimen 2	32μ	40μ
Specimen 3	20μ	40μ
Specimen 4	16μ	36μ
Specimen 5	16μ	32μ

In specimens 1 and 2, the oesophagus was shorter than in the other three. From the above it is clear that there is a gradual thickening of the muscle wall of the oesophagus in *P. cervi*, but it is not so marked as in the case of the other two species quoted above, and therefore it is not visible in whole specimens in *P. cervi*. Indeed, even in sectioned specimens it is difficult to appreciate with a low power, because the thickening is very gradual and not very marked, and it is only when the point is specially looked for with a high power that one realises its existence. In the collection of material from the *Cobus* sp. of antelope in the above list, some specimens were found which showed the oesophagus to be slightly bulbous even in whole worms, and on sectioning one of these the muscle wall of the oesophagus was found to be 20μ thick at the anterior and 72μ thick at its posterior end. Although the difference in this case is considerably greater than among the above specimens, the writer does not consider it sufficiently marked to be taken as a specific difference, and merely records it as a probable variant of *P. cervi*.

The intestine. Both the degree of convolution and the precise point of termination of the gut caeca are regarded by Fischoeder (1903) as important points in differentiating between species closely allied to *P. cervi*. These two points were examined in about 150 specimens cleared in carbolic and in about 40 sectioned specimens. Fifty specimens were taken from one bottle, which were chosen on account of their close similarity in external appearance, as it was thought by this means that variation due to artificial influences could be almost entirely eliminated. The result of the examination of these fifty specimens showed that the caeca were nearly straight in some cases and in others were distinctly convoluted, and

between these two extremes there were others with all degrees of convolution of the gut, which indicates that a variable species is being dealt with. This view was further supported by noting a different amount of convolution of the two caeca in the same worm in several instances. The point of termination of the two caeca also varied considerably; in some they ended quite clear of the anterior border of the posterior sucker, and in others they extended as far as the middle of the posterior sucker. In collection No. 10 of the above list, the worms were more fully extended than in any other collection, and in one of these specimens the gut was found to end 1·6 mm. in front of the anterior border of the posterior sucker. All gradations between the two extremes were seen, and in a few worms the caeca ended at different levels on the two sides. The remaining specimens which were not so uniform in size and shape were found to vary in the same way. It is also stated that the gut caeca terminate in a dorsally directed blind end. This was found to be so in most cases, but three specimens were seen in which the terminal part of the caeca ran ventrally. It is considered, as a result of these findings, that small variations in the gut caeca are not reliable points on which to separate species.

Posterior sucker. The ratio of the diameter of the posterior sucker to the length of the worm is used in many instances as a distinguishing feature. For the purpose of examining this character eighty-nine worms from one bottle were measured; all of the specimens used were fixed in a well-extended condition, differences in length from artificial causes such as shrinking being thus practically eliminated. In these eighty-nine specimens the ratio of the diameter of the posterior sucker to the length of worm varied greatly, all intermediate figures between 1 : 8 and 1 : 3·5 being found, and the variations were so gradual that it was found impossible to draw a dividing line at any point in the series. A large number of worms from other bottles were also measured in the same way, and as these were in different degrees of contraction the variation was found to be even greater than in the above series; in one strongly contracted example the ratio of sucker to length of worm was only 1 : 2·5. The collection obtained from England (No. 10) consisted of twelve specimens, which were part of the collection mentioned by Pillers (1922). They were all fully extended and presented a very uniform appearance externally, both in size and shape. Five of these specimens were cleared in carbolic and it was found that the diameter of the posterior sucker in proportion

to the length of the worm in the five specimens was as follows : 1 : 9·5, 1 : 7, 1 : 5·7, 1 : 5·4, 1 : 4·75. All of these specimens were gravid, and as they only varied from about 11 mm. to 10 mm. in length, it is obvious that the size of the sucker varies quite apart from the age and size of the worms. According to Pillers these worms are very uncommon in England, so it is improbable that more than one species is present in this small collection. All of these worms were cleared in carbolic acid, so that the general anatomical details were sufficiently clear to render it certain that only one species was being dealt with, and it was noted that the other characters used as distinguishing features between species of this group did not always occur in worms with a corresponding size of sucker. For instance, a worm with a sucker only one-eighth the length of the worm, and hence belonging to *P. gracile* on this account, might have its other diagnostic points more closely allied to *P. cervi* or any other of the species to be discussed below. This mixture of characters of more than one type could be indefinitely extended until the species became inextricably confused. It is considered, on account of the above observations, that the ratio of the posterior sucker to the length of the worm is much too variable to be used as a diagnostic character.

Genitalia. Testes. The size and shape of these organs and their relations to one another and to other structures were found to vary somewhat, depending on differences in age and on the degree of contraction in which the worms happened to be when fixed. One testis always lies behind the other, in or near the mid-line ; as a rule one testis is directly behind the other, but in young specimens with small testes they are sometimes slightly diagonally placed. When the testes have grown, however, this diagonal arrangement is no longer so obvious. Also in young worms, and even in fully extended examples with eggs in the uterus, there is sometimes a distinct interval between the two testes up to as much as 1 mm. In these instances the testes are circular in outline. On taking a series of worms of gradually increasing age and degree of contraction, the testes are found to come closer and closer together until they touch ; after this they tend to become flattened with consequent extension laterally and dorso-ventrally, so that they appear oblong in shape and their borders approach nearer the periphery ; in extreme cases they even cause a bulging of the external surface of the worm. The relation of the posterior testis to the posterior sucker also varies considerably ; in young worms and well-extended adults the hinder testis lies altogether in front of the posterior

sucker, but in worms not so well-extended the posterior border of the hinder testis reaches, or overlaps, the anterior border of the sucker. The testes are always divided into lobes, which are more distinct in young worms than in older ones. These conclusions are based on the examination of over forty specimens.

Another point of some interest was that three or four specimens were found with the uterus quite full of eggs and the testes small and indefinite. From this it seems possible that the testes atrophy after fulfilling their functions.

Vas deferens. Fischoeder (1903) made an arbitrary division of this organ into three principal parts dependent on the anatomical characters of each part, and as this division is very useful for purposes of description his nomenclature will be followed in the present paper.

The three portions of the vas deferens are as follows :—

1. Vesicula seminalis. = A thin-walled coiled tube formed by the junction of the two vasa efferentia.

2. Pars musculosa. = The continuation of the vesicula seminalis, furnished with a fairly thick muscular wall.

3. Pars prostatica. = A shorter portion of the tube surrounded by a collection of large cells, the prostatic cells.

The pars prostatica leads into a duct known as the Ductus Ejaculatorius, which unites with the termination of the uterus within the genital papilla, and which is known as the Ductus Hermaphroditicus.

The vesicula seminalis varies greatly in size and amount of convolution in different specimens. These variations depend on several factors, such as the degree of contraction of the worm, and whether the vesicula is empty, partly filled, or fully packed with spermatozoa when the worm is killed. The pars musculosa is liable to vary from the same causes, and to these must be added variation due to the state of contraction of its own muscular wall. The muscle wall of the pars musculosa is composed of two layers, an outer longitudinal layer, and an inner circular layer, so that contraction or relaxation of one or both of these layers can cause considerable variation in the length, diameter, amount of convolution of the duct, or the proportionate thickness of the two muscle layers. Both the vesicula seminalis and the pars musculosa coil freely on themselves and on each other, so that their relations with each other vary greatly in different specimens, and even in different sections of the same specimen. From examination of a large number of sectioned specimens,

the writer has come to the conclusion that the relations of the vesicula seminalis and the pars musculosa are so variable that it is not possible to assign any special type of relation between these two parts to any one species as Fischoeder does in several instances. The pars prostatica varies considerably in size and shape ; this fact is clearly brought out in Table II, which is compiled from the measurement of fifteen individuals cut in sagittal section and mounted serially ; the maximum measurements are given in every case.

Table II.

The length and breadth in microns of the pars prostatica of fifteen specimens of *P. cervi*.

	Length	Breadth		Length	Breadth
1	178	178	9	436	257
2	218	138	10	475	297
3	218	218	11	535	396
4	257	198	12	594	317
5	277	198	13	594	396
6	297	178	14	594	475
7	317	138	15	594	495
8	317	218			

This table shows a regular gradation of sizes from the smallest to the largest, and from this it is concluded that it is not possible to separate species on this character.

Genital pore. The genital pore usually lies about opposite the gut fork or a little behind it, the variation in position depending on the length and course of the oesophagus, which naturally has a direct effect on the position of the gut fork. But in one sectioned specimen which was fully grown and very much contracted, and in consequence only about 4 mm. in length, the genital pore was only 1/4·8 of the body length from the anterior end of the worm, and it lay opposite the junction of the middle and posterior thirds of the anterior sucker, that is, far in front of the gut fork. This is not in agreement with the statement of Fischoeder (1903) who, in his description of *P. cervi*, says:

'Die Geschlechtsöffnung liegt am hinten Ende des vordern Körperdrittels in der Höhe oder kurz hinter aber niemals vor der Gabelstelle der Darmschenkel.'

Genital atrium and genital papilla. These two structures are used extensively in specific diagnosis both by Fischoeder (1903) and by Stiles and Goldberger (1910). The papilla is composed almost wholly of muscle, and the atrium is surrounded by a special thickening of the subcuticular layer. Accordingly, both are liable to great alterations in form, and further, the papilla is capable of being withdrawn deeply into the body of the worm with resultant deepening of the genital atrium, or else it can be completely extruded through the genital pore, in which case the genital atrium disappears. As would be expected, many worms show intermediate stages between these two extremes. The variations of the genital apparatus are fully discussed in *P. explanatum* (see fig. 2), and as exactly the same type of variations are found in *P. cervi* the reader is referred to *P. explanatum*. The conclusions to be drawn from these facts are that the presence of one or two chambers in the genital atrium, or even the total absence of this cavity, or the size and shape of the genital papilla, are purely a matter of chance and are of no use in distinguishing various species. If Fischoeder's figures (1903) of *P. cervi* are referred to, it will be noted that in fig. 1 the pore is shown as a small cavity with no sign of the papilla protruding, whereas figs. 2 and 3 show the papilla protruded through the pore and there is no sign of an atrium at all. This suggests that Fischoeder recognised the possibility of variation in these structures without referring to it in the text. Small papillae are also described as being found on the internal surface of the genital atrium in some species and not in others. Examination of a number of sectioned specimens has shown that these papillae may be present or absent when the atrium is present, and a further fact which makes these structures still more liable to variation is that often the atrium itself is not present.

Ovary. It is recognised by all observers that the ovary may vary considerably in position in the same species. The present investigation fully bears this out.

Shell gland. This gland always lies close to the ovary, and so varies in company with it.

Vitellaria. The vitellaria appear to be the most variable of all the organs in *P. cervi*, and the number of gland groups, their size, and their distribution, undoubtedly increase considerably as age advances. But even when comparing them in over one hundred specimens in which the uterus was well filled with eggs they were found to show marked variation. Among these hundred odd specimens the anterior limits of the vitelline

glands were found to lie as far forward as the hinder end of the anterior sucker in some cases, whereas in others they did not reach as far as the genital pore, and between these two extremes all degrees were found. Sometimes in the same worm the glands did not reach the same level on both sides. The posterior limits of the vitellaria were also found to vary considerably, but as a general rule they ended a little behind the termination of the gut caeca ; but this was not invariably the case, for in a few instances the vitelline follicle groups were found extending to the extreme posterior end of the worm and could be made out in the parenchyma surrounding the opening of the posterior sucker. The degree of extension of the vitellaria inwards on the dorsal and ventral surfaces also varied markedly. In some cases the glands were strictly limited to the area outside the intestines on each side, and from this limited distribution all stages of inward extension were found up to a point where the glands of the two sides were practically continuous with one another all along both surfaces. It was noted, however, that when the glands were widely spread they seemed to be sparsely distributed, and when fairly circumscribed in their distribution the groups of follicles were much more closely gathered together. Fischoeder (1903) uses slight differences in distribution of the vitelline glands for specific diagnosis ; the writer does not consider this possible, because in his series of over one hundred gravid specimens of *P. cervi* a much greater range of variation was found than Fischoeder describes in his different species, and between the extreme limits of these variations all possible degrees were found which made it impossible to separate one species from another.

Uterus. The dorsal antero-posteriorly directed portion of the uterus is described as being more convoluted in some species than in others. After a large number of specimens were examined it was realised that the amount of convolution the uterus shows is subject to a wide range of variation in *P. cervi.* This variation was found to be dependent on two factors, first, the amount of contraction of the worm, and second, the number of eggs in the uterus. It was found that in specimens with no eggs the uterus was nearly straight, but as it became more and more filled with eggs the degree of convolution of the uterus increased also. It is therefore considered that slight differences in the amount of convolution of the uterus cannot be taken into consideration in specific diagnosis.

Eggs. Eggs taken from the uterus of several preserved specimens of *P. cervi* were found to vary considerably in size, being from 114μ in length

TABLE III.

Relations of the ovary, shell gland, Laurer's canal and excretory pore in five specimens of *P. cervi* cut in transverse section.

Specimen	A	B	C	D	E
Relations of ovary	To right of mid-line. In same dorso-ventral plane as shell gland. Midway between dorsal and ventral surfaces.	To left of mid-line, well towards ventral surface.	In mid-line ventro-posterior to shell gland. Slightly ventral of mid-transverse plane.	Well to right of mid-line in mid-transverse plane.	To left of mid-line: just ventral of mid-transverse plane. Just anterior to hinder border of testis.
Relations of shell gland... ...	In mid-line. Midway between dorsal and ventral surfaces.	Directly dorsal of ovary.	Just to right of mid-line and slightly dorsal of mid-transverse plane.	Just to right of mid-line antero-internal to ovary and just dorsal of mid-transverse plane.	Immediately dorsal of ovary.
Course of Laurer's canal ...	First dorsally to right of excretory bladder, then turns mesially and crosses to left side anterior to excretory bladder and posterior to excretory duct.	Dorsally and anteriorly on left side of excretory bladder	Dorsally and posteriorly on right side of excretory bladder.	Dorsally and anteriorly on right side of excretory bladder.	Dorsally and posteriorly on left side of excretory bladder
Opening of Laurer's canal ...	100μ posterior to and 300μ to left of excretory pore. 50μ anterior to anterior border of shell gland. 250μ anterior to base of ventral sucker.	75μ posterior and 400μ to the left of excretory pore. 500μ anterior to anterior border of shell gland. 325μ anterior to posterior border of hinder testis. 650μ anterior to base of ventral sucker.	500μ posterior and 625μ to right of excretory pore. 75μ posterior to posterior border of shell gland. On a level with base of ventral sucker.	200μ posterior and 300μ to right of excretory pore. Directly above shell gland. 175μ anterior to base of ventral sucker.	500μ posterior and 650μ to the left of excretory pore. 100μ posterior to posterior border of shell gland. On a level with base of ventral sucker.
Opening of Excretory pore ...	In mid-line 250μ anterior to anterior limit of excretory bladder, and 100μ posterior to posterior border of hinder testis.	In mid-line, 275μ anterior to anterior limit of excretory bladder. 400μ anterior to posterior border of hinder testis.	Slightly to left of mid-line, and vertically above left side of excretory bladder. 75μ posterior to anterior limit of excretory bladder. On a level with posterior border of testis.	In mid-line 100μ posterior to anterior limit of excretory bladder. 75μ posterior to posterior border of hinder testis.	Slightly to right of mid-line and vertically above right side of excretory bladder. 125μ posterior to anterior limit of excretory bladder. 375μ anterior to posterior border of hinder testis.

by 60μ in breadth up to 176μ in length by 90μ in breadth. The above figures represent the extremes of size found after several specimens had been dissected ; the maximum variation in eggs from a single worm was only about 10μ in length and 7μ in breadth. It is considered on this account that small differences in size of the eggs are not reliable characters for specific diagnosis, at all events when the eggs are taken from the uterus of preserved specimens. It is possible they may be more uniform in size after being laid.

Laurer's canal. The relations between Laurer's canal, the excretory bladder, and excretory pore are used by Fischoeder (1903) in distinguishing certain species from one another. He evidently attaches great importance to these relations, because he divides the genus *Paramphistomum* into three groups on these characters, and Stiles and Goldberger (1910) have followed Fischoeder in this respect. Fischoeder's three groups of the genus are as follows :—

1. Laurer's canal crosses the excretory bladder completely. This means that Laurer's canal opens behind the excretory pore and in the mid-line of the worm.

2. Laurer's canal and the excretory bladder do not cross. This means that Laurer's canal opens in front of the excretory pore.

3. Laurer's canal crosses the excretory bladder incompletely. This means that Laurer's canal opens behind the excretory pore, but to the side of the mid-line, i.e., the same side as that on which the shell gland lies.

On account of the importance attached to these characters, five specimens of *P. cervi* all from one bottle and as nearly as possible similar in external appearance, were cut in transverse sections, 25μ thick, and mounted serially, so as to test the value of the above statements. The result of this investigation is shown in Table III.

Table III and Fig. 1, which consists of camera lucida drawings of Specimens A, B, and C in Table III, show that the relations of Laurer's canal to the excretory bladder and excretory pore vary considerably, and in some cases the relations of these structures do not come under any of Fischoeder's three headings. It is probable that if more worms were examined still other arrangements would be found, but it was considered that the above five specimens sufficiently proved that these relations were unreliable for diagnostic purposes. Although only the above five specimens are included in the table, about thirty others were examined ; these were cut in sagittal and coronal sections and the above results were thereby

TABLE IV.

Summarising the chief characters described by Fischoeder (1903 and

	(1) *P. cervi*	(2) *P. gracile*	(3) *P. microbothrium*
Length	5-12 mm.	11-15 mm.	8-11 mm.
Shape	Posterior end rounded	Almost cylindrical.	Somewhat more flattened dorso-ventrally than *P. cervi*. Only slightly curved ventrally like *P. gracile*.
Relation of sucker to length of worm	1 : 4 to 1 : 5	1 : 8	1 : 4 to 1 : 5.
Genital pore	Opposite or behind gut fork, $\frac{1}{3}$ of body length from anterior end.	Well behind gut fork, $\frac{3}{4}$ of body length from anterior end.	Behind gut fork, $\frac{2}{3}$ of body length from anterior end. Has a genital atrium and more definite muscular sphincter.
Vesicula seminalis	Very broad, thin walled, in front of anterior testis.	Round canal in median plane.	Lies dorsal of pars musculosa, unlike *P. cervi* and *P. gracile* where it is behind.
Pars musculosa	0·8 to 1·0 mm. long, walls 18μ to 22μ thick, varying with its degree of fulness. Straight or slightly coiled.	500μ to 600μ long, walls 18μ to 22μ thick. Moderately straight.	Walls 45μ-50μ thick: almost exclusively consisting of circular muscle, with a single outer layer of longitudinal muscle. Strongly coiled.
Pars prostatica	300μ to 600μ in transverse diameter.	500μ to 600μ long by 250μ to 350μ broad.	500μ to 600μ long.
Papillae on anterior end and in 'pharynx'	On external surface and in anterior portion of 'pharynx.'	On external surface and not in 'pharynx.'	Not mentioned.
Termination of gut caeca	Dorsal to sucker.	Anterior to sucker.	Anterior to sucker.
Testes	ANTERIOR: 2 mm. to 2·8 mm. dorso-ventral. 1·5 mm. to 2 mm. transverse. POSTERIOR: 2·8 mm. to 3·5 mm. dorso-ventral. 1 mm. to 1·5 mm. transverse. Slightly on opposite sides of middle line, close to ventral surface.	ANTERIOR: Oval, 1·2 mm. by 0·7 mm. slightly dorsal. POSTERIOR: More rounded, 0·9 mm. by 1·0 mm., slightly ventral. Only lie slightly on opposite sides of median line.	ANTERIOR: 2·3 mm. to 2·5 mm. dorso-ventral. 1·5 mm. to 1·7 mm. transverse and longitudinal. POSTERIOR: As a rule somewhat larger. A little more markedly on opposite sides of mid-line. Close to ventral surface.
Vitellaria	Extend from 'Pharynx' to posterior border of ventral sucker. Extend on dorsal and ventral surfaces. In coarse groups of follicles close together.	Extend from hinder border of 'Pharynx' to anterior border of sucker; do not extend markedly on dorsal and ventral surfaces. In five groups of follicles somewhat irregularly placed.	On one side reach from posterior border of 'Pharynx' to anterior border of sucker. On other side from gut fork to middle of sucker. Similar to *P. cervi* in size and extent on dorsal and ventral surfaces.
Ovary	Close behind base of sucker, either to right or left of mid-line.	Behind posterior testis either to left or right of mid-line and slightly towards ventral surface (i.e., anterior to sucker).	Further from mid-line than in *P. cervi* and *P. gracile*.
Uterus	Portion dorsal of testes markedly wavy.	Portion dorsal of testes wavy.	As in *P. cervi*.
Eggs	145-156μ × 75-82μ.	115-125μ × 72-80μ.	145-150μ × 75-80μ.
Laurer's canal	Runs dorsally towards anterior and opens in mid-line about level of posterior border of the hinder testis. 1-1·2 mm. behind excretory pore.	Curves posteriorly and opens at level of anterior border of ovary 1·5 mm. behind excretory pore.	Opens to one side and not in mid-line: opposite anterior border of ovary. 250-300μ behind excretory pore.
Excretory bladder	Flask-shaped, close to dorsal surface. Pore opens anteriorly in mid-line. Crossed by Laurer's canal at junction of anterior and middle third.	Further from dorsal surface. Pore on a level with hinder border of posterior testis. Is crossed by Laurer's canal about its centre.	Similar to *P. cervi*. Pore in mid-line. Laurer's canal does not cross bladder

* At the first glance *P. bothriophoron* appears to have several rather marked points of difference from the other five species, e.g., pars musculosa is closely coiled; the pars prostatica is very long; and the genital atrium is large and deep, with no genital papilla. musculosa.

1904), as typical of the following six species of *Paramphistomum*.

(4) *P. epiclitum*	(5) *P. bathycotyle*	(6) *P. bothriophoron**
5-9 mm.	11-15 mm.	6-9 mm.
Anterior $\frac{1}{4}$ strongly curved ventrally, the remainder straight, as in *P. cervi*: the greatest circumference at 2nd and 3rd thirds.	Ventrally curved. Greatest transverse diameter near posterior end.	Of 4 specimens: 2 strongly curved; 2 slightly curved ventrally.
1 : 3 to 1 : 4·5.	*In text* $\frac{2}{5}$ of body length; *in drawing* $\frac{1}{3}$ of body length.	Not mentioned.
At junction of 1st and middle thirds. Well behind gut fork.	About middle of anterior third behind gut fork. Very little muscle surrounds it.	In middle of anterior half. Not so well developed as in other species. Like *P. microbothrium*.
Dorsal and posterior of pars musculosa.	Lies loosely coiled between intestines.	Behind and not dorsal to pars musculosa. Closely coiled.
Walls 18μ to 22μ thick.	Ventral and anterior to vesicula, 0·6-0·75 mm. long and 18μ-22μ thick, not coiled.	Strongly developed, not loosely coiled like others, but closely coiled. Muscle different from all other species because, unlike those which have nearly all circular muscle surrounding a single layer of longitudinal, the longitudinal is nearly as thick as the circular.
Unlike *P. cervi*, which is round, it is long 600μ-800μ, and 250μ-300μ thick.	Almost globular, 400μ-500μ in diameter.	1·0-1·2 mm. long.
Not mentioned.	On both external surface and in 'pharynx.'	Not seen.
Beside base of sucker.	Close in front of anterior border of sucker.	Close in front of sucker.
Anterior: 1·8-2·2 mm. dorso-ventral. 0·9-1·2 mm. longitudinal. 1·2-1·6 mm. transverse. Posterior: 2·1-2·5 mm. dorso-ventral. 0·7-0·1 (? 1·0) mm. longitudinal 1·6-2·0 mm. transverse.	Longitudinal: 1·0-1·3 mm. } Both about Dorso-ventral: 1·5-1·8 mm. } same. Like *P. cervi*, slightly out of mid-line.	Anterior: 0·7-0·8 mm. long. Posterior: 0·8-1·0 mm. long. Both 2·0-2·3 mm. dorso-ventral. More deeply lobed. Lie in similar position to *P. microbothrium*.
Of different sizes and very irregular, but close together. Stretch from beginning of oesophagus as far as base of sucker. Reach further on dorsal and ventral surfaces than in *P. cervi*.	Almost from 'pharynx' to opposite anterior border of sucker. Almost confined to lateral fields only slightly encroaching on dorsal and ventral surfaces. Single follicles small.	From gut fork almost to middle of sucker and also extend on dorsal and ventral surfaces.
Between posterior testis and base of sucker and on same side of mid-line as anterior testis.	Similar position to *P. cervi*, but only slightly away from mid-line.	Between hinder testis and sucker very near ventral surface and very much to the side.
As in *P. cervi*.	Broad and filled with eggs.	Filled with eggs.
145-155μ × 75-80μ.	115-125μ × 70-75μ.	125-135μ × 65-70μ.
Opens in mid-line about the level of its origin.	Runs directly dorsal and opens on a level with ovary.	Opens opposite side to ovary as in *P. microbothrium*, opposite anterior border of shell gland.
Opens on level of middle of posterior testis 500-600μ anterior to Laurer's canal. Laurer's canal crosses between 1st and 2nd thirds of bladder.	Different from *P. cervi* because it has a long anteriorly directed canal which opens opposite posterior border of anterior testis about middle of length of worm.	More rounded; in front of sucker near dorsal surface.

The longitudinal muscle of the pars musculosa is said to be very thick, and it is figured as being arranged in distinct columns; the It is conceivable that all of these conditions could be caused by one factor, viz., contraction of the longitudinal muscle of the pars

confirmed. But one point was found to be of value in regard to the relations of the opening of Laurer's canal and the excretory pore, and that is, that in species in which Laurer's canal is described as opening behind the excretory pore this is invariably the case, although the actual distance at which one pore lies behind the other varies a good deal.

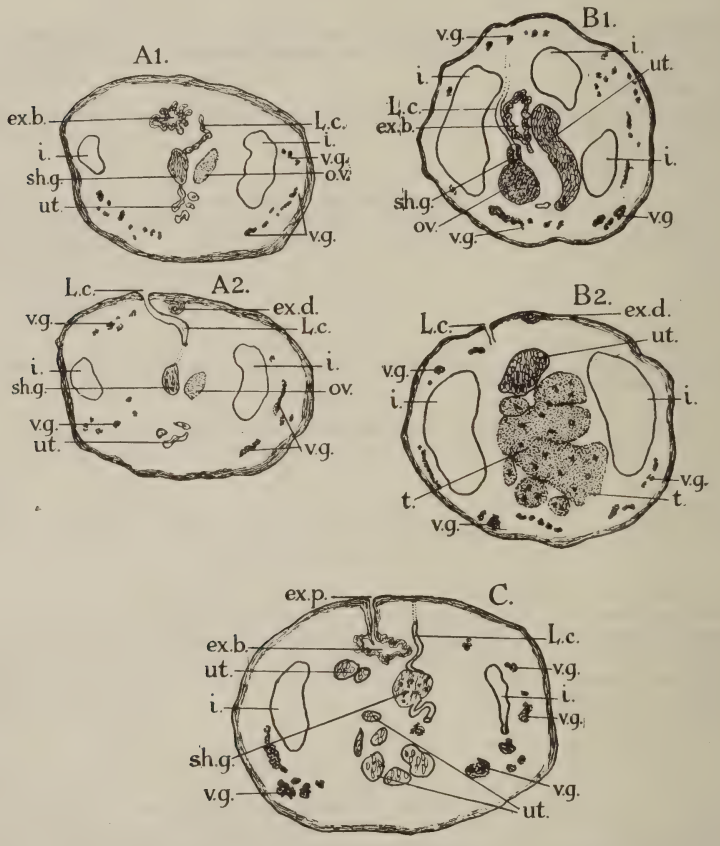

Fig. 1. *Paramphistomum cervi*, transverse sections. *A*1 and *A*2—Sections of one specimen at different levels. *B*1 and *B*2—Sections of a second specimen at different levels. *C*—Section of a third specimen passing through the excretory pore. *ex.b.*—excretory bladder; *ex.d.*—excretory duct; *ex.p.*—excretory pore; *i.*—intestine; *L.c.*—Laurer's canal; *ov.*—ovary; *sh.g.*—shell gland; *ut.*—uterus; *v.g.*—vitelline gland. × 16.

Careful examination of the descriptions of the six species of *Paramphistomum* in Table IV shows that the differences between them are very minute, and a further comparison of this table with the results of the extensive examination that has been carried out by the writer shows that with one exception all the characters used by Fischoeder as of specific

value in distinguishing these six species come within the range of what appear to be variations of *P. cervi*. The exception is the length of the pars prostatica ; the maximum length observed by the writer for this organ was 594μ, whereas Fischoeder gives it as 800μ in *P. epiclitum*, and 1·2 mm. in *P. bothriophoron*. But this is such a small difference that the author is inclined to disregard it and to consider it probable that the above species are all one.

There are three species, *P. papilligerum*, *P. papillosum* and *P. indicum*, described by Stiles and Goldberger (1910) which also appear to the writer to be identical with *P. cervi*.

Paramphistomum papilligerum, Stiles and Goldberger, 1910.

Apparently the material on which this species was made consisted of a series of frontal sections loaned to the authors by Shipley.

The only difference between this species and *P. cervi* is that *P. papilligerum* is described as having small papillae on the inner surface of the genital atrium. It has already been shown that papillae in this position may be present or absent in *P. cervi*, and further that the presence of a genital atrium itself is a variable character. There is hence no justification for the separation of this species.

Paramphistomum papillosum, Stiles and Goldberger, 1910.

The material at the disposal of the authors is stated to be a single non-gravid specimen. The species is characterised by the presence of papillae on the anterior end of the worm, in the oral cavity, and in the genital atrium. Papillae in these positions have already been shown to be variable characters of *P. cervi*, being present or absent in one or all of these positions. In *P. papillosum* the excretory pore is stated to be dorsal to the excretory bladder, and in *P. papilligerum* it is stated to be anterior to the bladder ; but it must be remembered that in their descriptions of these two worms, Stiles and Goldberger state that eggs were present in the uterus of *P. papilligerum*, and not in *P. papillosum*. It has already been shown that in *P. cervi* the excretory pore appears to pass forward as age advances ; this fact is in agreement with the difference observed between the species *P. papilligerum* and *P. papillosum*, when it is remembered that the former is probably a younger specimen than the latter. It is therefore considered that *P. papillosum* is synonymous with *P. cervi*.

Paramphistomum indicum, Stiles and Goldberger, 1910.

These worms were said to have been found in two bottles along with other species, and no eggs were observed. The description and drawings of this species by Stiles and Goldberger agree in all points with many specimens of young *P. cervi* without eggs in the uterus, examined by the writer during the course of the present investigation. That the writer's specimens referred to were young *P. cervi* and not another species is rendered practically certain, because in the same bottles worms in all stages of development could be found, so that the gradual change from worms similar in appearance to *P. indicum* could be followed in a long series until a typical *P. cervi* with uterus full of eggs could be found. The writer is therefore of the opinion that on its present description *P. indicum* cannot be distinguished from young *P. cervi*.

In the writer's opinion *P. gracile*, *P. bothriophoron*, *P. microbothrium*, *P. epiclitum*, *P. bathycotyle*, *P. papilligerum*, *P. papillosum* and *P. indicum* are all synonyms of *P. cervi*.

Paramphistomum liorchis, Fischoeder, 1901.

This worm is easily distinguished from other species of the genus, because the testes are not lobed ; it is the only species in which the testes exhibit this character, and in which Laurer's canal opens behind the excretory pore.

Paramphistomum pisum, Leiper, 1910.

The material available for examination consisted of two collections, about thirty specimens in all. The location of the parasite in the host (Hippopotamus) is not given, but Leiper (1910) states that it is found in the small intestine.

No gravid specimens were observed by the writer, but about half the material was sexually mature. Leiper says the worms are pisiform when fresh and somewhat contracted when preserved. This agrees with the writer's observations for all of his specimens were slightly contracted with the result that they were almost globular in shape, the largest measuring about 3mm. in diameter. The worms agreed essentially with Leiper's description; both suckers communicate with the exterior by narrow canals as Leiper figures them, but in three specimens which the writer

sectioned the anterior sucker was found immediately beneath the cuticle as in other members of the genus, and is not separated from it by a canal of considerable length as in Leiper's fig. 34.

Paramphistomum gigantocotyle, Brandes, 1896.

Host :—*Hippopotamus amphibius*. Location :—Stomach. Locality :—Africa.

As the original description of this species is somewhat inadequate, Leiper (1910) redescribed it.

According to Leiper this worm may be distinguished from other species by the relative size of the posterior sucker and by the shape of the testes. Although the diameter of the posterior sucker given by Leiper, viz., 3·2 mm. in a worm of 8 mm. in length, is relatively great, it is not by itself a sufficient character for identification, because in *P. explanatum* the relative size of the sucker is often more than this. But the testes appear to be characteristic, as although they are placed one behind the other as in *P. cervi*, they differ in that whilst in the latter they are divided into several lobes, in *P. gigantocotyle* each testis is nearly completely divided into two portions by a deep transverse groove, so that in sections they may appear as four organs, a condition that is never found in *P. cervi*.

Paramphistomum explanatum (Creplin, 1847), Fischoeder, 1901.

Synonymy :—

Paramphistomum calicophorum, Fischoeder, 1901.
Paramphistomum crassum, Stiles and Goldberger, 1910.
Paramphistomum cauliorchis, Stiles and Goldberger, 1910.
Paramphistomum fraternum, Stiles and Goldberger, 1910.
Paramphistomum siamense, Stiles and Goldberger, 1910.

The material available consisted of the following collections :—

1. One bottle from the stomach of a bullock killed at Durban, South Africa.
2. One bottle from the stomach of a bullock killed at Townsville, Australia. (Over 100 specimens.)
3. One bottle from the stomach of a bullock killed at Khartoum, Sudan.
4. One bottle from the stomach of a bullock killed at Blantyre, Nyasaland.
5. Two bottles from the stomachs of two hartebeests shot near Blantyre, Nyasaland. (Both bottles contained over 100 specimens.)

This worm is readily distinguished from *P. cervi*, because the testes are always diagonally situated one overlapping the other, both laterally and antero-posteriorly, in fully grown worms, whereas in full-grown *P. cervi*, the testes lie one directly behind the other.

At the beginning of his decription of *P. explanatum*, Fischoeder (1904) states that this species most nearly resembles *P. bathycotyle* on account of its shape, and throughout his paper he contrasts these two species without reference to others. Plate VI, fig. A, clearly indicates that shape cannot be taken as a diagnostic point ; the fact that the testes in *P. bathycotyle* are placed antero-posteriorly at once distinguishes it from *P. explanatum* and renders further comparison of these species unnecessary. Apart from the arrangement of the testes the general anatomy of *P. explanatum* is very similar to that of *P. cervi* and the organs are found to be subject to similar variations. The relatively large size of the posterior sucker is apparently regarded as an important point in distinguishing between *P. explanatum* and other species. Fischoeder (1904) states that the worm varies from 8 mm. to 13 mm. in length. But in giving the size of the posterior sucker, he makes use of a single specimen 8 mm. in length and gives no particulars of the dimensions of the suckers in larger worms. The sucker of this single specimen is stated to be 3·5 mm. in its antero-posterior diameter, and 3·0 mm. transversely. That is, the greatest diameter of the sucker to the length of the worm is as 1 : 2·3.

Ten specimens of this worm were cleared in carbolic acid and the length of the worms and the size of the posterior sucker ascertained ; these are given in Table V.

TABLE V.

Measurements of ten specimens of *P. explanatum*.

Specimen	Length of worm in mm.	Diameter of sucker in mm.	Ratio of diameter of sucker to length of worm
1	10·1	3·5 × 2·4	1 : 2·9
2	9·7	2·8 × 2·8	1 : 3·6
3	9·6	2·9 × 2·7	1 : 3·3
4	8·5	3·6 × 3·6	1 : 2·4
5	7·6	3·8 × 3·8	1 : 2·0
6	6·8	3·5 × 3·5	1 : 1·9
7	6·8	3·5 × 3·5	1 : 1·9
8	6·6	2·6 × 2·6	1 : 2·5
9	6·6	2·6 × 2·6	1 : 2·5
10	6·0	2·6 × 2·6	1 : 2·3

Average 1 : 2·5

From this table it is clear that the ratio of the diameter of the sucker to the length of the worm varies above and below Fischoeder's figure, which closely approximates to the mean. On the whole the sucker is relatively larger than in *P. cervi*, but it is subject to such variations that it cannot be taken as an absolute guide in diagnosis. Another point brought out in the above table is that the sucker is not typically oval in shape, being only occasionally met with in this form.

In describing the genital apparatus, Fischoeder states :

'Auch das genitalatrium ist nur sehr klein. Die dasselbe umgebende, von dem übrigen Körperparenchym wenig abgegrenzte Musculatum ist nur 0·08-0·1 mm. stark, und die in Grunde des Atriums befindliche Papille ist ebenfalls nur äusserst schwach entwickelt.'

Fig. 2 consists of drawings of the genital apparatus of three specimens of *P. explanatum* cut in sagittal section and from these it is clear that such a precise description as the above of this portion of the worm is not permissible. Although this point has not been figured in detail in dealing with other species, the same range of variation in the genital papilla and genital atrium has been found. Fischoeder says that the genital organs are displaced towards the anterior end of the worm, and that the testes reach near to the ventral surface, in which case they are of necessity in front of the posterior sucker. In some instances this is correct, as is illustrated in fig. 3, but that the testes are not invariably in this position is shown in figs. 4 and 5. In these two worms the testes are seen drawn away from the ventral surface and they lie dorsal to the posterior sucker. In all probability this is an artificial condition brought about by contraction of the worms at the time of fixation, because both these specimens were noted to be considerably contracted before they were cut, and this is borne out by the irregularity of outline shown in the drawings on the dorso-posterior portion of the worm, especially in the case of fig. 4. This probability is further supported by the appearance in fig. 6, which is a sagittal section of an immature well-extended specimen ; in this drawing the single testis figured is seen to be situated nearer to the ventral than to the dorsal surface and well in front of the sucker. The four worms from which the above drawings were made all came from the same bottle, and specimens in varying degrees of contraction and of different ages are found with characters intermediate between them.

If fig. 6 is examined, it will be readily understood how by contraction of the worm the testes are brought to the position seen in the two previous

figures. The base of the posterior sucker slopes backwards and towards the dorsal surface, and antero-posterior contraction of the worm would cause the testes to impinge on this surface, when they would follow the path of

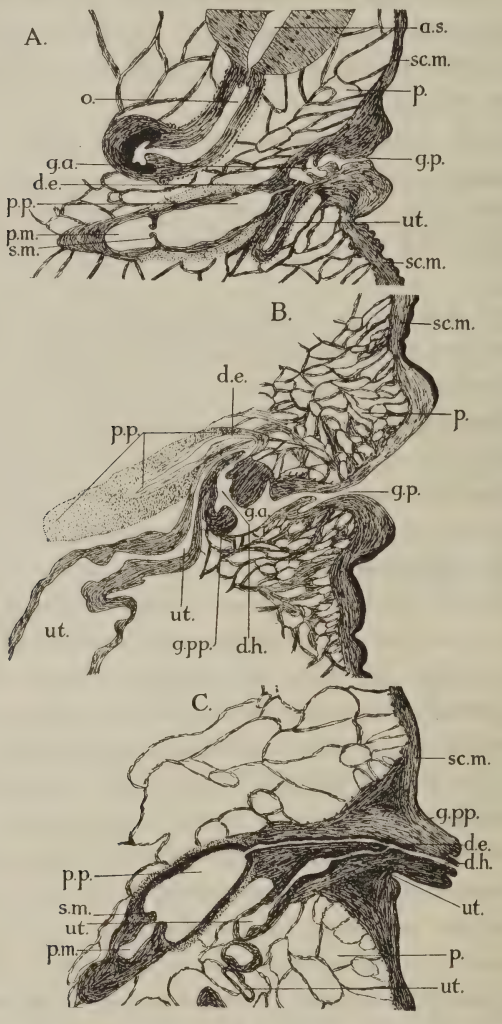

Fig. 2. *Paramphistomum explanatum.* Sagittal section through the genital pore of three specimens. *A*—Genital papilla fully retracted. *B*—Genital papilla partly retracted. *C*—Genital papilla fully extruded. *a.s.*—anterior sucker; *d.e.*—ductus ejaculatorius; *d.h.*—ductus hermaphroditicus; *g.a.*—genital atrium; *g.p.*—genital pore; *g.pp.*—genital papilla; *p.*—parenchyma; *p.m.*—pars musculosa; *p.p.*—pars prostatica; *sc.m.*—subcuticular muscle; *s.m.*—sphincter muscle; *ut.*—uterus. × 30.

least resistance and pass up towards the dorsal surface of the worm; at the same time the testes exert some pressure on the sucker, causing its base to

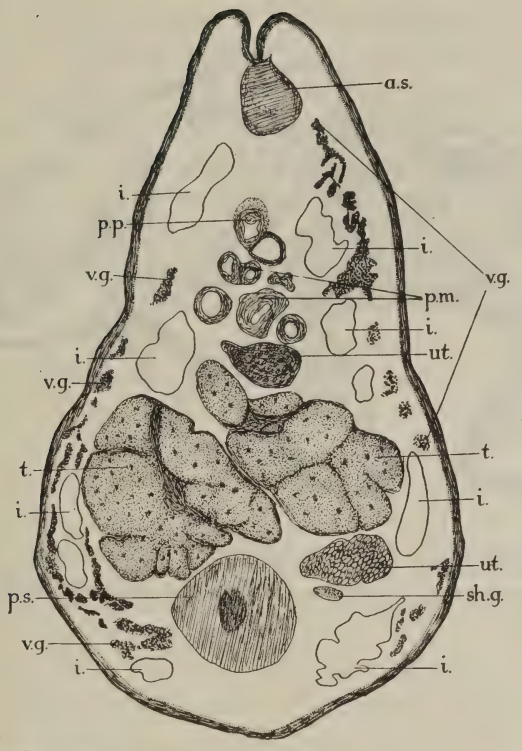

Fig. 3. *Paramphistomum explanatum.* Coronal section of gravid worm. *a.s.*—anterior sucker; *i.*—intestine; *p.m.*—pars musculosa; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland. × 12.

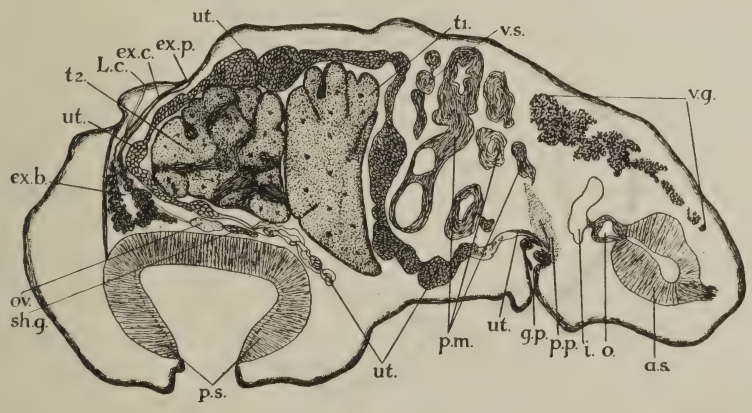

Fig. 4. *Paramphistomum explanatum.* Sagittal section of gravid worm near the mid-line. *a.s.*—anterior sucker; *ex.b.*—excretory bladder; *ex.c.*—excretory canal; *ex.p.*—excretory pore; *g.p.*—genital pore; *i.*—intestine; *L.c.*—Laurer's canal; *o.*—oesophagus; *ov.*—ovary; *p.m.*—pars musculosa; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t1.*—anterior testis; *t2.*—posterior testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.s.*—vesicula seminalis.

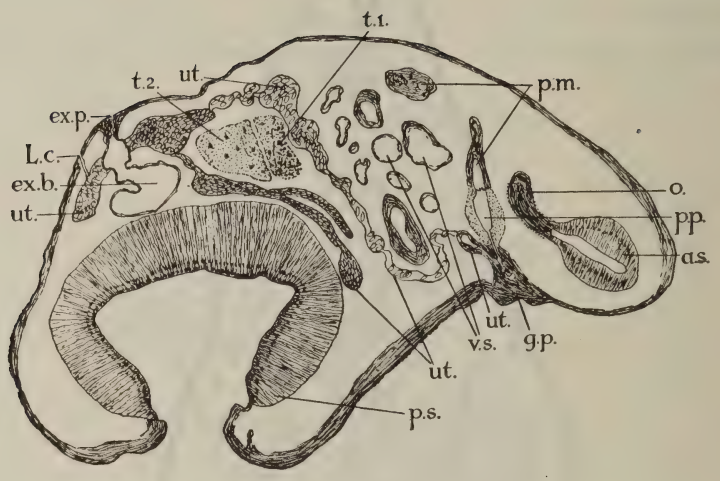

Fig. 5. *Paramphistomum explanatum.* Sagittal section of a partly gravid worm near the mid-line. *a.s.*—anterior sucker; *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *g.p.*—genital pore; *L.c.*—Laurer's canal; *o.*—oesophagus; *p.m.*—pars musculosa; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *t1.*—anterior testis; *t2.*—posterior testis; *ut.*—uterus; *v.s.*—vesicula seminalis. × 16.

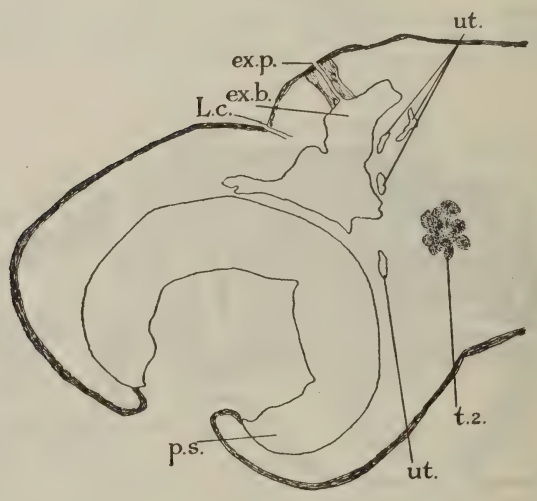

Fig. 6. *Paramphistomum explanatum.* Sagittal section of posterior part of immature worm near the mid-line. *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *L.c.*—Laurer's canal; *p.s.*—posterior sucker; *t2.*—posterior testis; *ut.*—uterus. × 16.

become flatter and also making it look almost directly ventrally instead of ventro-posteriorly. The full effect of such contraction is shown in fig. 4.

Fischoeder describes and figures the excretory bladder as being of a long flask-shaped type with a narrow canal running forward from it and opening about the level of the posterior border of the hinder testis. Figs. 4, 5 and 6 clearly show that the shape of the excretory bladder is very variable and that the excretory canal runs dorsally from the bladder in young worms, whereas in older specimens it becomes longer and narrower until, in fully gravid worms, it opens well in front of the bladder.

Stiles and Goldberger (1910), in their diagnostic key of the genus, place *P. explanatum* in the group of worms with the 'excretory pore prevesicular,' and in the sub-group in which the following characters are given as diagnostic. 'Testicular fields median, coincide or overlap, zones lobate, testes much smaller than and near the acetabulum; ventral chamber absent; excretory vesicle long and narrow; acetabulum very large.' Their specific definition is: 'Genital pore in postbifurcal zone; musculosa (?); prostatica longer than musculosa; acetabulum less than half as long as body; caeca sinuous, moderately broad, end in acetabular zone; body 8 to 13 mm. long; type host *Bos indicus* at Berlin, Germany.'

With the exception of the relation of the testes to each other all of the above diagnostic characters are so variable that none of them are reliable.

Paramphistomum calicophorum, Fischoeder, 1901.

At the outset of his description of this worm Fischoeder (1903) states:

'Das Material ist stark geschrumpft, brüchig; trotz Behandlung mit Iod und Wochen langer Einwirkung von Kreosot lässt sich eine zur Untersuchung von Totalpräparaten nur wenig brauchbare Durchsichtigkeit erzielen, mit Ausnahme von einzelnen unreifen Exemplaren aus dem letzt genannten Glase. Dagegen ist es mir gelungen, von reifen Exemplaren aus dem Glase No. F. 659 für meine Zwecke einiger Maassen brauchbare Schnittserien anzufertigen.'

From this it is obvious that he was unable to obtain a dorso-ventral view of a gravid worm, and, from his figures, he was apparently content with sagittal and transverse sections of full-grown specimens; the only dorso-ventral view he gives is that of an immature worm with the testes small and wide apart, an arrangement which is quite unlike that found in gravid specimens. Thus he failed to realise the identical arrangement of the testes in mature *P. explanatum* and *P. calicophorum*.

As the sucker is said to be somewhat smaller in relation to the length

of the worm in *P. calicophorum* than it is in *P. explanatum*, Table VI has been drawn up by utilising the figures given by Fischoeder for these two worms and by summarising the figures in Table V of the present paper.

Table VI.

	P. explanatum, Fischoeder	*P. calicophorum*	*P. explanatum.* Present material summarised from Table V
Length of worm	8 mm. to 13 mm.	10 mm. to 15 mm.	6 mm. to 10·1 mm.
No. of worms measured	?	?	10
Diameter of posterior sucker	3·5 mm. × 3 mm.	3 mm. to 4·3 mm.	2.6 mm. to 3.8 mm.
No. of suckers measured	1	?	10
Ratio of diameter of sucker to length of worm expressed in fractions ...	$\frac{1}{2{\cdot}3}$	'Not quite $\frac{1}{3}$'	From $\frac{1}{2{\cdot}9}$ to $\frac{1}{3{\cdot}6}$

It seems clear from Table VI that there is no constant relation between the diameter of the posterior sucker and the length of the worm.

Fischoeder recognises the variation to which the genital apparatus is liable and to which attention has been drawn in *P. explanatum* in the present paper. He states :

'Die Hoden liegen fast neben einander (fig. 29, 30), der vordere etwa in der Mitte des Körpers, mehr dorsal, der hintere mehr ventral (fig. 29, 30 u. 31).'

His references to the figures prove that he is describing the appearance in young worms, which is borne out by his later statement :

'Bei reifen Individuen nehmen sie die ganze, stark verdickte, hintere Hälfte des Thieres bis dicht zum Saugnapfe ein (fig. 31).'

He makes no reference to the appearance of the testes in immature *P. explanatum.*

With regard to the excretory bladder, excretory pore, and Laurer's canal, he says :

'In Bezug auf das Lageverhältniss des Excretionporus und der Ausmündungsstelle des Laurer'schen Canals sind häufig gewisse Differenzen zwischen den reifen

und unreifen Individuen zu verzeichnen, die jedoch auch mit der Entwicklung der Hoden und der damit verbundenen stärkern Ausdehnung der hintern Körperpartie in Verbindung gebracht werden können. Während nämlich bei den unreifen Thieren in der Regel die Excretionsblase etwa im Niveau des vordern Randes des Saugnapfes und der Laurer'sche Canal nur 0·3-0·4 mm. dahinter ausmündet (fig. 30, 32 u. Textfig. E), befindet sich der Excretionsporus bei den geschlechtsreifen Thieren meist viel weiter nach vorn, fast in der Höhe des vordern Randes des hintern Hodens (fig. 31), und die Ausmündung des Laurer'schen Canals 0·6-0·8 mm. hinter dem Excretionsporus.'

It is therefore clear that in the case of *P. calicophorum*, Fischoeder recognised the variation to which these structures are liable, but, as he does not seem to have made a detailed examination of immature material of other species, he failed to realise the general application of this fact. While in agreement with Fischoeder in the first part of his statement, viz., that the excretory pore and excretory canal vary in their relations to the bladder as age advances, the writer cannot support the latter part of his statement that Laurer's canal opens further behind the excretory pore in gravid worms than it does in immature examples. While agreeing that a variation in this particular can be found, it has been observed to be quite independent of age.

Stiles and Goldberger (1910), apparently basing their definition of *P. calicophorum* on Fischoeder's paper (1903), write :

'Excretory pore prevesicular.

Testicular fields separate, not median, zones overlap, testes lobate, much smaller than acetabulum ; . . . excretory vesicle not narrow but swollen . . .'

From this it is clear they have ignored Fischoeder's statements quoted above and have taken their definition mainly from characters found in immature worms, which makes it useless for diagnostic purposes. Careful comparison of Fischoeder's descriptions of the species *P. explanatum* and *P. calicophorum* thus indicates that there are no differences between them, except such as may be explained by differences in age and normal variation ; therefore *P. calicophorum* is a synonym of the species *P. explanatum*.

Paramphistomum crassum, Stiles and Goldberger, 1910.

Stiles and Goldberger (1910) made the species *P. crassum* from three specimens which they state were found with other forms in a bottle from India, the host being *Bos indicus*. They also say that no eggs were seen, so it is probable their material was immature.

On comparing Stiles and Goldberger's description of *P. crassum* with

Fischoeder's description of *P. calicophorum*, the only essential difference between the two is that the excretory pore is dorsal to the excretory bladder in *P. crassum*, and although Stiles and Goldberger assert that the excretory pore is anterior to the excretory bladder in *P. calicophorum*, it is not in agreement with Fischoeder's statement, quoted above, except in fully grown worms, and with this the writer's observations are in agreement. It has also been shown that in *P. explanatum* without eggs the excretory pore is in the position ascribed to it in *P. crassum* by Stiles and Goldberger. When it is recalled that the latter observers saw no eggs it seems probable that they were dealing with young specimens of the same species as Fischoeder.

Paramphistomum cauliorchis, Stiles and Goldberger, 1910.

In describing this species Stiles and Goldberger (1910) state that they found three specimens in one bottle and four in another. The host was *Bos indicus* and eggs were not observed. In their description of *P. crassum* Stiles and Goldberger say that it closely resembles *P. cauliorchis;* this is fully borne out by comparing the two descriptions. There is this difference, however, viz., that in *P. cauliorchis* they state that Laurer's canal ' opens slightly to right or left of the median line, 60 to 320μ cephalad of the excretory pore,' whereas in *P. crassum* Laurer's canal is described as opening ' about 0·72 mm. caudad of the excretory pore.' But there is some doubt as to the correctness of their observations in *P. cauliorchis* for two reasons. First, in their fig. 62, which is a side view of *P. cauliorchis*, they show Laurer's canal running parallel with the excretory canal, and although the termination of Laurer's canal is not actually shown, it looks as if it would open about the same level as the excretory pore. In the second place, they have made a subgenus *Cauliorchis* of which *P. cauliorchis* is the type, and in their definition of this Stiles and Goldberger state that Laurer's canal is ' caudad to caudo-laterad of the excretory pore, the two pores may be close together . . .' Another point is that in *P. crassum* the testicular fields are said to overlap and in *P. cauliorchis* they are said to be separate, in the latter case being similar to their definition of *P. calicophorum*. The writer has already shown, and is confirmed by Fischoeder, that in *P. explanatum* the testes are separate in young worms, and that in older ones the fields overlap, and although age is the most important factor in influencing this difference, the writer has found young worms of the same

type with the testes overlapping. It is accordingly concluded that there is no difference between *P. crassum* and *P. cauliorchis*.

Paramphistomum fraternum, Stiles and Goldberger, 1910.

Railliet, Henry and Bauche (1914a) state that *P. fraternum* is a synonym of *P. explanatum*, and they advance such good reasons for their opinion that it is proposed to accept their conclusion without further discussion. In the light of the present investigation, however, there is one point of interest in the conclusion of Railliet, Henry and Bauche, viz.: Stiles and Goldberger give as one of the distinguishing characters of *P. fraternum* that the genital pore is opposite the anterior sucker in this species, whereas it is posterior to the gut fork in *P. explanatum*. From this it may be inferred that the above observers do not consider the position of the genital pore of any specific value. Moreover, Fischoeder also seems to be of the same opinion, as Railliet, Henry and Bauche state that they obtained his opinion on their material and that he agreed with them as to its identity.

Paramphistomum siamense, Stiles and Goldberger, 1910.

This species is differentiated from *P. cervi* and *P. fraternum* by Stiles and Goldberger and is therefore considered new by them. In their diagnosis from *P. cervi* they use the difference in size of the posterior sucker, the position of the genital pore, and the shape of the worm, but they omit the one real distinguishing character, viz., the different relations of the testes to one another in the two species. *P. fraternum* is distinguished from *P. siamense* because the worms are said to be of different shape; the posterior sucker of *P. fraternum* is relatively smaller than it is in *P. siamense*; and the genital pore is proportionately nearer to the anterior end in *P. fraternum*. The question of small differences in shape may be disposed of by reference to Plate VI, fig. A, which is a photograph of several specimens of *P. explanatum*, all of which were taken from one bottle. With regard to the position of the genital pore, this is given as midway between that described for *P. explanatum* and *P. fraternum*, so the opinion of Railliet, Henry and Bauche (1914a), which has been quoted under the discussion of the latter species, is of equal value in this case. The relative difference in size of the posterior sucker in the two species requires more careful consideration.

In the case of *P. fraternum* Stiles and Goldberger say that only two specimens ' in poor condition ' were available. The length of one of these specimens is given as 9·75 mm. when in alcohol, and the same worm measured 8·94 mm. after imbedding and sectioning. The diameter of the posterior sucker of this worm is given as 3·75 mm. in the antero-posterior and 3·25 mm. in the transverse direction. In the case of *P. siamense* the length of two specimens measured in glycerine alcohol is given as about 6 mm. and 9 mm. respectively. The posterior sucker was measured in three ' press preparations,' and the diameters of the three are given as 3·5 mm., 4 mm. and 5 mm. respectively. It is not quite clear what is meant by press preparations, but it is assumed that it means that the worms were subject to pressure before being measured. The writer has found that pressure between slides is of great advantage in obtaining a clear view of the internal organs in carbolic cleared specimens, but it was found that measurements of worms while under pressure in this way were quite unreliable. This was especially the case with regard to the posterior sucker ; because, the worms being much thicker at the posterior end than at the anterior end, when pressure was applied a considerable flattening and consequent distortion was caused to the hinder end before the anterior end was subject to any pressure. It is therefore clear that the posterior portions of the worms were always relatively more distorted than the anterior portions, and hence the structures in the hinder part were relatively greatly increased. It seems that this is what has occurred in Stiles and Goldberger's case, and that owing to their failure to recognise this fact they have been led to ascribe specific value to what is in reality an artificial condition. Indeed, from the writer's experience, it is surprising that the difference in size of the posterior sucker in *P. fraternum* and *P. siamense* was not greater than they actually found it. It is therefore considered that the small difference between the relative size of the posterior suckers in these two worms should not be regarded as of any diagnostic value, and in consequence the two worms are probably identical with one another and with *P. explanatum*.

As a result of the above investigation it is concluded that *P. calicophorum*, *P. crassum*, *P. cauliorchis*, *P. fraternum* and *P. siamense* are all synonyms of *P. explanatum*.

In all the species of the genus *Paramphistomum* that have been dealt with up to the present the excretory pore is anterior to the opening of

Laurer's canal. Those species in which Laurer's canal opens anterior to the excretory pore will now be considered. As the writer has not had an opportunity of examining the following species, the description is in each case summarised from the original.*

Paramphistomum orthocoelium, Fischoeder, 1901.

SYNONYMY :—

Paramphistomum dicranocoelium, Fischoeder, 1901.
Paramphistomum streptocoelium, Fischoeder, 1901.
Paramphistomum scoliocoelium, Fischoeder, 1904.
Paramphistomum parvipapillatum, Stiles and Goldberger, 1910.
Paramphistomum shipleyi, Stiles and Goldberger, 1910.

First found in the stomach of a *Bos kerabau* that died in Berlin.

The following notes were made from Fischoeder (1903).

Cuticular papillae are present on the anterior end of the worm. The oesophagus is twice as long as the anterior sucker, and is surrounded by especially large and numerous cells. The muscle walls of the oesophagus are not thicker than in most other species of the genus. The gut caeca are not wavy, they are almost straight ; they lie about midway between the dorsal and ventral surfaces on each side of the worm, and they end 0·5 mm. to 1 mm. in front of the posterior sucker ; the diameter of the caeca is 1 mm. to 1·2 mm. dorso-ventrally and 500μ to 600μ transversely. With the exception of the vitellaria, the genital organs exhibit no special characters. The genital papilla is well developed and almost fills the atrium, being in most cases slightly protruded. The testes lie one behind the other in the posterior half of the worm ; their size and the distinctness of their lobulation is variable. The vitellaria, however, consist of round to oval groups of follicles nearly uniform in size and about 300μ in diameter. As a rule, they are in a single row along the ventral border of the caeca on each side except near the posterior end, where they are grouped to the number of from four to six follicle groups which lie close together. The eggs are 105μ to 115μ in length by 60μ to 65μ in breadth.

* Since completion of this paper the writer has been able to examine a collection of a few specimens of *P. orthocoelium* from the stomach of a sheep at Hong Kong. Although it is necessary to section the worm to see the course of Laurer's canal, simple clearing in carbolic acid is sufficient to establish the identity of this species. It is easily distinguished from *P. cervi* by the vitellaria which in this species are arranged in very numerous comparatively small groups of follicles, never more than about 300μ in diameter, and showing a marked tendency to encroach on the dorsal and ventral surfaces of the worm, whereas in *P. orthocoelium* the number of follicle groups is much less, they are considerably larger, being up to 700μ in diameter, and are limited almost exclusively to the lateral fields external to the gut caeca.

Paramphistomum dicranocoelium, Fischoeder, 1901.

The following notes are taken from Fischoeder (1903).

Cuticular papillae were not seen on the anterior end. The worm is very near *P. orthocoelium*. The oesophagus is shorter than in *P. orthocoelium*, its extreme length being one and a half times as long as the anterior sucker, and instead of becoming thicker only quite close to the gut fork the muscle wall commences to thicken about its middle, and from this point it gradually increases from 20μ to 60μ-75μ at the posterior end. The gut caeca are straight, but differ from *P. orthocoelium* in being nearer to the dorsal than to the ventral surface, and they are only 250μ to 350μ in diameter; they end about 1 mm. in front of the posterior sucker. The genital papilla is well developed, but is strongly retracted as a rule. The vesicula seminalis is larger and lies farther towards the dorsal surface, but on the other hand the pars musculosa is not so long as it is in *P. orthocoelium*, and the pars prostatica is somewhat shorter also. The ductus hermaphroditicus may be a fairly broad pear-shaped cavity, or, when the papilla is strongly retracted, the ductus hermaphroditicus may be merged in the genital atrium. The vitelline glands are arranged in similar groups to *P. orthocoelium*, but they lie as a rule in two rows and show no special grouping behind the ends of the caeca. The anterior ends of the vitellaria may be at different levels. The other genital organs are approximately the same as in *P. orthocoelium*. The eggs measure 145μ to 150μ in length and 75μ to 80μ in breadth.

Paramphistomum streptocoelium, Fischoeder, 1901.

The following notes were taken from Fischoeder (1903).

Cuticular papillae are present on the anterior end as in the case of *P. orthocoelium*. The oesophagus is only about as long as the anterior sucker; the gut caeca are strongly convoluted and end opposite the base of the sucker. The genital atrium is distinguished by the presence of a ring-like prominence on its inner wall which divides the atrium into a small ventral and a large dorsal chamber. The testes, as well as varying in proportion to the size of the worm, may be of different sizes in the same worm and apparent partial atrophy of these organs has been noted in gravid worms. The position of the testes is the same as in *P. dicranocoelium*. The female genitalia only show slight differences from the previous species.

The vitellaria are confined to the sides of the worm and stretch from the gut fork in front to the posterior sucker behind as in *P. dicranocoelium*. The follicles are in groups similar to the previous species (300μ to 700μ in diameter), but they are present in larger numbers and are therefore smaller in size than in the two previously named species. The anterior limits of the vitellaria may be at different levels on the two sides as in *P. dicranocoelium*. The eggs measure 105μ to 115μ in length by 60μ to 65μ in breadth.

Paramphistomum scoliocoelium, Fischoeder, 1904.

The following notes are taken from Fischoeder (1904).

The musculature of the oesophagus is similar to that of *P. dicranocoelium*. The gut caeca are less wavy than in *P. streptocoelium*, and they are nearer to the dorsal than the ventral surface as in *P. dicranocoelium*. The genital pore is, as a rule, widely open. The vitellaria are similar to the other three species ; they are composed of coarse follicles which lie at the sides of and external to the gut fork, from about the level of the gut fork in front opposite to the base of the posterior sucker behind. The eggs are 135μ to 145μ in length by 65μ to 75μ in breadth.

Paramphistomum parvipapillatum, Stiles and Goldberger, 1910.

The following notes are taken from Stiles and Goldberger (1910).

The cavity of the anterior sucker is furnished with moderate sized papillae. The oesophagus is estimated to be not shorter than the anterior sucker and it exhibits a thickening of its muscle wall in its posterior half. The gut caeca are slightly wavy and end opposite the middle of the posterior sucker. The genital papilla is embraced by a ring-like collar beset with fine papillae. External to this collar is another ring marked off from it by a groove, and this groove may not be present in cases with the genital papilla protruded. The vitellaria consist of numerous follicles lying at the sides of, above, and below the gut caeca ; the gland groups on the left-hand side extend slightly further inwards than those on the right hand. The vitellaria extend from the hinder end of the anterior sucker in front to opposite about the middle of the posterior sucker behind. The eggs measure 135μ in length by 67μ in diameter.

Paramphistomum shipleyi, Stiles and Goldberger, 1910.

The following notes are from Stiles and Goldberger (1910).

The entrance to the anterior sucker and the sucker itself are lined with small papillae. The muscle wall of the oesophagus begins to grow thicker from about the middle of its length towards the posterior end. The caeca are wavy and end about the level of the anterior border of the cavity of the posterior sucker. The diameter of the gut varies at different points along its course, there being marked dilatations followed by constrictions. The testes are one behind the other, but overlap slightly. The ductus ejaculatorius opens close to, but separate from and just above the opening of the uterus. The space into which this ductus ejaculatorius opens is narrow and slit-like.

'From this space a short duct passes ventrad and may be regarded as piercing the axial region of a mushroom-like structure (figs. 123-126) to open into another slit-like atrium somewhat larger, however, than the one into which the male and female ducts open. A duct about 30μ in diameter leads from this atrium and apparently pierces a stout conical papilla, which may be regarded as the genital papilla, to open into a small genital atrium which connects with the exterior by the genital pore.'

The eggs are about 135μ in length by 71μ in breadth.

In conducting a critical examination into the specific value of the above worms, it is in the first place obvious that the points used for differentiation are all similar to those used in the group of worms that have been considered under *P. cervi*. But in the present instance the differences are even smaller than in the former case. For instance, differences in length of the oesophagus greater than in these six species have been noted in all species examined. This is only to be expected when it is realised that the oesophagus is well supplied with muscle, contraction or relaxation of which can easily account for the differences observed. The amount of increase of thickness towards the posterior end of this organ has also been shown to vary considerably in the same species. The point of termination of the gut caeca only varies very slightly in the above six species; this character has been found to have no diagnostic value in other cases, and that in the present instance it is equally valueless is appreciated, when it is noticed that in *P. scoliocoelium*, Fischoeder shows the caeca ending in front of the posterior sucker in fig. 7, and behind the anterior border of the sucker in fig. 8, although in the text he says they end in front of the posterior sucker. It has also been found that slight differences in the

amount of convolution of the caeca, as well as in their diameter, are of no value. A moment's reflection will explain the reason for this. The caeca are hollow structures furnished with muscular walls and it will thus be obvious that the amount of convolution may vary considerably according to the degree of contraction of the muscular wall of the gut and of the whole worm. The value of slight differences in the distribution of the vitellaria has also been shown of no use for specific diagnosis in all other species, and that the same probably applies in the present series of worms is borne out by comparison of the statements in regard to these glands in several of the worms. For example, Fischoeder states that *as a rule* the gland groups are in a single row in *P. orthocoelium*, and in *P. dicranocoelium* they are *as a rule* arranged in a double row ; but as he does not state what the exceptions to these rules are, all that one can infer is that the vitellaria are subject to some variation in both these species and cannot, therefore, be accorded any specific value. The liability to variation of the vitellaria is also borne out by the following contradictory statements by the same author : in *P. orthocoelium* he says the vitelline follicles are in groups, each of which is about 300μ in diameter ; in *P. dicranocoelium* they are similar to the above ; and in *P. streptocoelium* they are in groups similar to the above, but having a diameter of from 300μ to 700μ ; whilst in the next sentence he states they are present in *P. streptocoelium* in far greater numbers than in the two previous species and are in consequence of smaller size. With regard to the genital apparatus, there is no evidence of any allowance having been made for variations due to protrusion or retraction of the genital papilla at the time of fixation. For example, the genital papilla of *P. orthocoelium* is described as being well developed and in most cases filling the atrium ; in *P. dicranocoelium* the papilla is said to be well developed and in most cases strongly retracted ; in *P. streptocoelium* a ring-like prominence divides the atrium into two chambers ; in *P. scoliocoelium* the genital pore is stated to be, as a rule, widely open ; in *P. parvipapillatum*, the papilla is described as being embraced by a collar-like ring beset with minute papillae ; and in *P. shipleyi* from the single sectioned example that they studied Stiles and Goldberger describe a complicated arrangement of ducts and atria which, in the writer's opinion, is only a description of a worm with the papilla in strong retraction such as can be found in any species, if enough specimens are examined. All the various descriptions of genital papilla and atrium described above have been seen by the writer in all the species in which he has had sufficient

material to examine a long series of mature worms. It is therefore considered that in the present case they cannot be regarded as specific differences. The only other point relates to the small papillae described as present in some species of this group of worms and absent in others. In all other species they have been found to vary to a great extent, and accordingly it is probable that in the present case they vary in the same way, and are in consequence of no diagnostic value.

The evidence obtained from the examination of closely allied species, indicates that the points on which the differentiation of the above six species is based, are of no specific value. It is therefore considered that *P. dicranocoelium*, *P. streptocoelium*, *P. scoliocoelium*, *P. parvipapillatum* and *P. shipleyi* are synonyms of *P. orthocoelium*.

Paramphistomum buxifrons, Leiper, 1910.

Host :—*Hippopotamus* sp. Location :—Stomach. Locality :—Uganda.

According to Leiper, *P. buxifrons* can readily be distinguished by its leaf-like shape which resembles the leaf of a box tree. A large example may measure 5 mm. in length by 3 mm. in breadth and be only 0·4 mm. in thickness at the middle of the body. The testes also are a distinguishing character, as they are not lobed and they are placed diagonally in the posterior part of the worm. The only other species with testes not lobed are *P. liorchis* and *P. wagandi* and in these cases the testes are one behind the other, but in *P. buxifrons*, Laurer's canal opens in front of the excretory pore, whilst in *P. liorchis* Laurer's canal is behind the excretory pore.

Paramphistomum wagandi, Leiper, 1910.

Host :—*Hippopotamus* sp. Location :—Stomach. Locality :—Uganda.

The testes are not lobed and are placed one behind the other, and the worm is therefore very similar to *P. liorchis ;* the two species are distinguished by the position of the opening of Laurer's canal, which is in front of the excretory pore in *P. wagandi*, whereas it is behind the excretory pore in *P. liorchis*.

In his descriptions of these species, Leiper lays down by definite measurement the exact size and position of the various organs, the appearance of the genital atrium, the shape of the excretory bladder, etc. In most cases these particulars appear to have been obtained from a single sectioned

specimen. This fact is considered unfortunate, because slight variations from the data given by Leiper are almost certain to be found when these worms are more fully known, and will in all probability lead to a multiplication of species on the ground of slight variations, in the same way as seems to have occurred in most of the other species.

Genus *Cotylophoron*, Stiles and Goldberger, 1910.

Definition.—Paramphistominae : with a genital sucker distinctly marked off from the subcuticular muscle layer.

Type species : *Cotylophoron cotylophorum* (Fischoeder, 1901), Stiles and Goldberger, 1910.

Key to Species

Laurer's canal opens posterior to excretory pore	*C. cotylophorum*
Laurer's canal opens anterior to excretory pore	*C. minutum*

Cotylophoron cotylophorum (Fischoeder, 1901), Stiles and Goldberger, 1910.

Synonymy :—

Paramphistomum cotylophorum, Fischoeder, 1901.
Cotylophoron indicum, Stiles and Goldberger, 1910.

First found in the stomach and intestines of *Bos* sp. in German East Africa.

The material available for study in the present case consisted of the following collections :—

1. Ten bottles from the stomach of bullocks killed at Sierra Leone, West Africa.
2. Four bottles from the stomach of buffaloes (*Bubalus* sp.) in the Upper Shire River, Nyasaland.
3. Four bottles from the stomach of two nswala (*Aepyceros melampus*) shot in the Upper Shire River District, Nyasaland.
4. One bottle from the stomach of a Pagan dwarf bull from Ilorin, Northern Nigeria.
5. One bottle from the stomach of a waterbuck (*Cobus* sp.) from Zeref, Khartoum.
6. One bottle from the stomach of a hartebeest (*Bubalis* sp.) from Nyasaland.
7. One bottle from the stomach of an antelope sp. (?) from Rhodesia.
8. One bottle from the stomach of an antelope sp. (?) from Nyasaland.

In some of these bottles were many hundreds of specimens, so ample material was available for examination.

The species *C. indicum* was made by Stiles and Goldberger from six specimens. They state that no eggs were observed, so it is probable that their material was immature.

The differences between *C. cotylophorum* and *C. indicum* are summarised by Stiles and Goldberger as follows :—

'*Cotylophoron indicum* comes close to *C. cotylophorum* from which it differs chiefly in the structure of the oesophagus, which is provided with a bulbous thickening in the latter species, but is without it in the former. The two differ also in the details of structure of the copulatory apparatus and in the position of the genital pore. In *C. indicum* the genital sucker is less sharply delimited, projects less, has a much smaller genital atrium, and the genital pore is decidedly postbifurcal ; on the other hand, in *C. cotylophorum* the genital sucker is sharply marked, with rim prominently bulging the venter, with a relatively roomy genital atrium and with the genital pore in the bifurcal zone.'

It should be noted that Fischoeder (1903) in his description of *P. cotylophorum* says the uterus is strongly convoluted and it is full of eggs, showing that his specimens were mature.

The following points have been worked out from the examination of many specimens of *C. cotylophorum,* either cut and mounted in serial sections, or examined whole in carbolic acid.

Oesophagus. In their description of the species *C. indicum,* Stiles and Goldberger state that the walls of the oesophagus are thick, but give no other important details of its characters. In the present instance, the thickness of the muscle walls of the oesophagus, as well as its length and direction, were found to be very variable. Although it was much more distinct in some cases than in others, there was always a gradual increase in thickness of the muscle wall of the oesophagus from the anterior end towards the posterior end, in exactly the same way as described in *P. cervi.* Fig. 7 represents camera lucida drawings of eleven specimens of *C. cotylophorum* cut in sagittal section. It will be noted that in figs. K and A, the oesophagus is approximately the same as in fig. 45 by Stiles and Goldberger, which is a drawing of *C. indicum.* Now, taking the remaining drawings in fig. 7 in the following order D, B, E, C, L, F, G, H, it will be observed that as the oesophagus gradually increases in length, its posterior extremity becomes more and more bulbous. Drawings C, L, G, and F are very similar to Fischoeder's fig. 38, which is a drawing of *C. cotylophorum* cut in the sagittal plane. In addition to the above characters it was noted that the worms from which drawings J and K were made contained no eggs, whilst all the others had eggs in the uterus, those with the longest oesophagus having the most eggs. It seems probable, therefore, that the differences in the oesophagus in *C. indicum* and *C. cotylophorum* are really due to differences of age. This is all the more likely when it is remembered that Stiles and Goldberger's material did not contain eggs,

and that Fischoeder's did. Two other points are also well illustrated in the above series of drawings, viz., that the course and length of the oesophagus are subject to considerable variation, and that the position of the genital pore varies in relation to the gut fork to such an extent that neither of these points is reliable for distinguishing between *C. indicum* and *C. cotylophorum*.

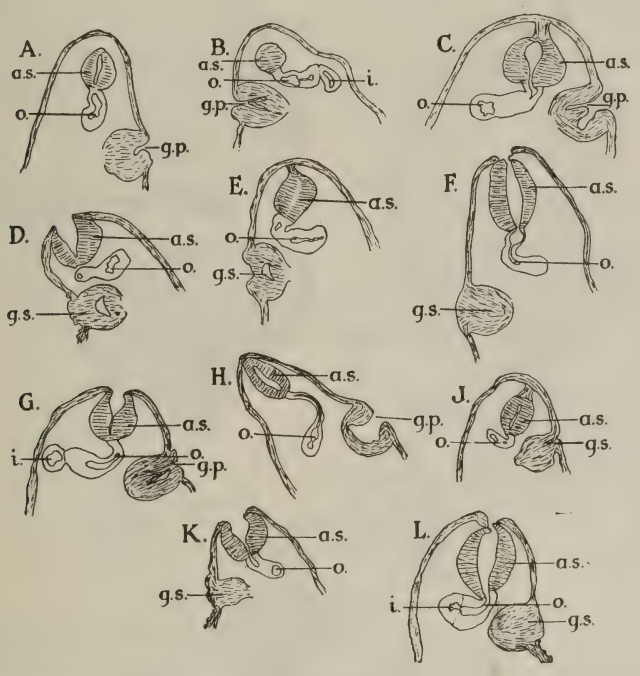

Fig. 7. *Cotylopboron cotylophorum*. Sagittal section through the anterior ends of eleven specimens to show oesophagus and genital sucker. *a.s.*—anterior sucker; *g.p.*—genital pore; *g.s.*—genital sucker; *i.*—intestine; *o.*—oesophagus. × 12.

Genital apparatus. The differences in size, and degree of extrusion or retraction of the genital sucker and papilla, with consequent changes in the genital atrium, were almost as numerous as the specimens examined. This is well illustrated in figs. 8 and 9, which show such great differences in appearance that at first sight it might be considered that different species are being dealt with, but it should be borne in mind that the above examples have been chosen for this very reason, and that many specimens have been examined showing all possible intermediate stages, thus rendering it practically certain that it is only a question of individual variation. Fig. 8, A1 and A2, are two sections of the same worm, and they have been included because Fischoeder (1903) states that the male and female

openings have always been noted by him to be separate in *C. cotylophorum* as shown in fig. 8, A2, which closely corresponds to Fischoeder's fig. 38. But a later section (fig. 8, A1) showed the male and female ducts uniting within the substance of the genital papilla and opening on the surface by

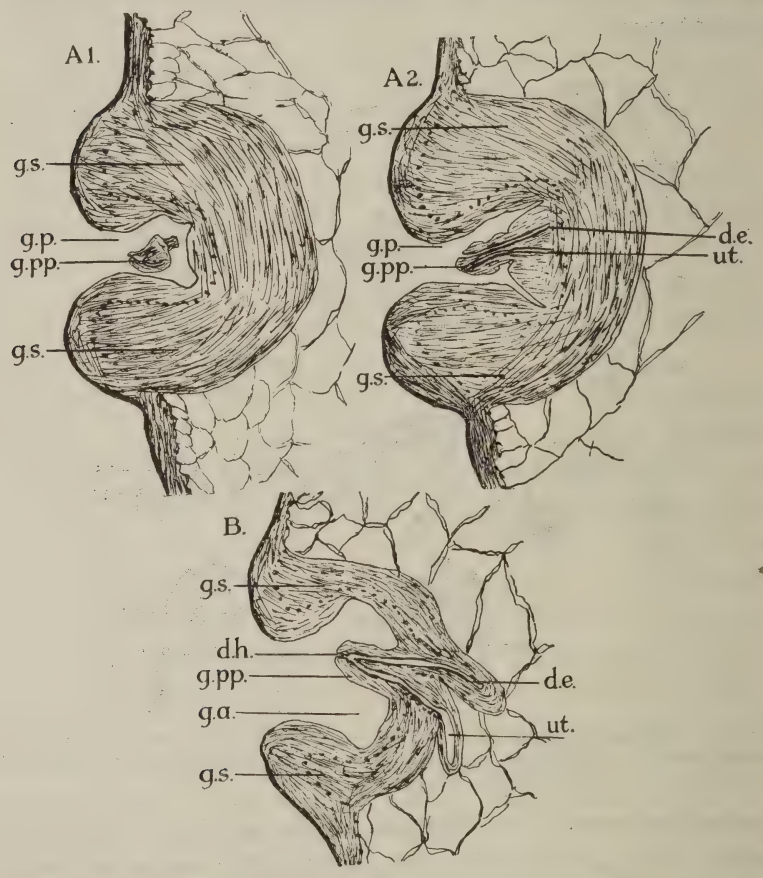

Fig. 8. *Cotylophoron cotylophorum.* Sagittal section through genital sucker. *A1*—Section showing tip of genital papilla with male and female ducts uniting in the usual way. *A2*—Section of same specimen with male and female ducts apparently opening separately, because tip of genital papilla is not seen. *B*—Section of another specimen with genital papilla lying in a wide atrium. *d.e.*—ductus ejaculatorius; *d.h.*—ductus hermaphroditicus; *g.a.*—genital atrium; *g.p.*—genital pore; *g.pp.*—genital papilla; *g.s.*—genital sucker; *ut.*—uterus. × 45.

a common duct. It is therefore clear that there is no essential difference between this worm and other species with regard to the termination of the male and female ducts. Fig. 8, B, shows how different the genital pore may appear if it is relaxed with a large genital atrium and a patulous genital pore, but in sections not passing through the genital atrium the genital

sucker was seen to be just as thick as in other cases. In fig. 9 two very different appearances of the genital apparatus are shown. Fig. 9, A, shows the whole genital sucker protruded beyond the surface of the worm, while in 9, B, it is deeply retracted within the body. It will be noted that in fig. 9, A, the subcuticular muscle extends past the base of the genital sucker in an apparently unbroken column of fibres, and that even opposite its base the parenchyma is not sharply marked off from the sucker, whereas in fig. 9, B, with the sucker deeply retracted, this separation is quite distinct. The conclusions drawn from these two figures are that the demarcation of

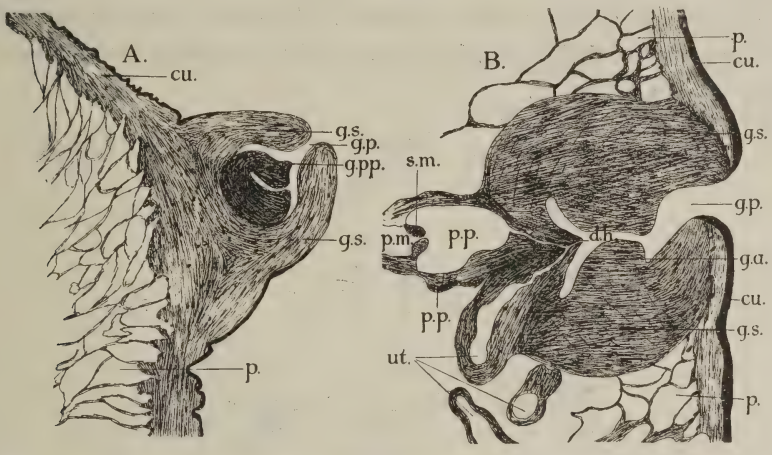

Fig. 9. *Cotylopboron cotylophorum.* Sagittal section of genital sucker of two specimens. *A*—Genital sucker fully extruded. × 40. *B*—Genital sucker fully retracted × 60. *cu.*—cuticle; *d.h.*—ductus hermaphroditicus; *g.a.*—genital atrium; *g.p.*—genital pore; *g.pp.*—genital papilla; *g.s.*—genital sucker; *p.*—parenchyma; *p.m.*—pars musculosa; *p.p.*—pars prostatica; *s.m.*—sphincter muscle; *ut.*—uterus.

the sucker from the parenchyma in this genus is more apparent than real, and that it is probably brought about by the subcuticular muscle and the contiguous parenchyma being stretched around the sucker when it is in the retracted condition. Comparison of the shape and size of the atrium in the drawings will make it sufficiently clear without further comment that minute descriptions of the shape and number of chambers in this atrium cannot be regarded as of any value for specific diagnosis. The conclusion arrived at from consideration of the above facts is that Stiles and Goldberger, in describing *C. indicum* from six specimens with no eggs in the uterus, were in all probability dealing with immature specimens of *C. cotylophorum.*

In addition to the above points, which were worked out in sectioned

specimens, evidence of variation in other characters was obtained by the examination of eighty specimens cleared in carbolic acid and examined whole.

Gut caeca. The amount of convolution of the caeca varies considerably, as does their point of termination. The most usual position for the caeca to end in this species is about opposite the middle of the posterior sucker, but in some cases they both ended well in front of the anterior border of the posterior sucker, and in others they nearly reached the posterior border of the posterior sucker. Between these two extremes all intermediate stages were found, and in a few instances the caeca on the two sides of the same worm ended at different levels. As a rule, the final turn of the caeca was directed dorsally, but this was by no means invariable, as is clearly shown in fig. 10.

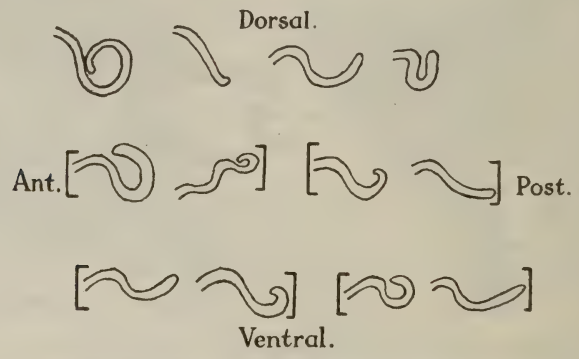

Fig. 10. *Cotylophoron cotylophorum.* Drawings showing various forms of termination of gut caeca. These figures are not to scale, and are drawn from carbolic cleared specimens. Those in brackets are the two caeca from a single worm.

Vitellaria. The distribution of the vitelline glands and the number of collections of follicles in each gland showed a remarkable degree of variation. The glands commenced anteriorly anywhere from about opposite the middle of the anterior sucker to a point slightly behind the genital pore ; as a rule, they extended posteriorly to a little behind the termination of the gut caeca, but in a few instances groups of follicles were seen extending to the extreme posterior end of the worm and surrounding the opening of the posterior sucker in the same way as in all the other species examined. The degree of extension inwards on the dorsal and ventral surfaces varied in the same way as in *P. cervi.* The anterior extension of the gland was often different on the two sides, and in one extreme instance the vitelline gland on the left side began in front of the genital pore and ended just

behind the corresponding caecum, but on the other side the gland was composed of a closely packed collection of follicle groups, which commenced opposite about the middle of the hinder testis and ended opposite the middle of the posterior sucker, being completely confined to the outer side of the caecum of that side.

Excretory system. The change in the relations of the excretory pore to the excretory bladder in worms of different age, which has been referred to in *P. cervi* and *P. explanatum*, has been found to hold good in the case of *C. cotylophorum*. This is clearly shown in fig. 11, which represents the

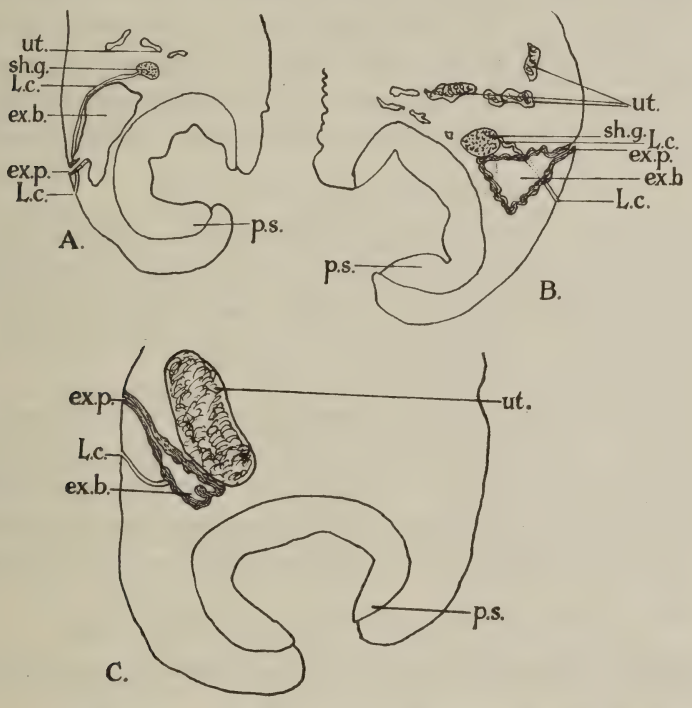

FIG. 11. *Cotylophoron cotylophorum.* Sagittal sections of three specimens near the mid-line to show the alteration in course of the excretory canal with increasing age. *A*—Immature worm. *B*—Partly gravid worm. *C*—More fully gravid worm. *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *L.c.*—Laurer's canal; *p.s.*—posterior sucker; *sh.g.*—shell gland; *ut.*—uterus. × 18.

posterior ends of three specimens of *C. cotylophorum* of different ages. These figures also emphasise the fact that the shape of the excretory bladder is very variable.

Leiper (1910) described two worms from the hippopotamus, which he placed in the genus *Paramphistomum*, but both of them have a definitely

delimited muscular thickening round the genital pore. Following the classification of Stiles and Goldberger (1910) these worms should be placed in the genus *Cotylophoron*. The following are the principal distinguishing features of these species according to Leiper's descriptions.

Cotylophoron minutum (Leiper, 1910).

Synonymy :—*Cotylophoron sellsi* (Leiper, 1910).

First found in the stomach of a *Hippopotamus* sp. in Uganda.

The testes are small and oval in shape and they lie one behind the other, being separated by a space about equal to the diameter of a single testis. Although it is not stated in the text, it is assumed from Leiper's fig. 32 that the testes are not lobed. Laurer's canal opens in front of the excretory pore. This worm differs from *C. cotylophorum* in that the testes are not lobed and that Laurer's canal opens in front of the excretory pore.

Cotylophoron sellsi (Leiper, 1910).

This species is very similar to *C. minutum*, the only differences being that it is larger and that its testes are relatively larger and closer together than in *C. minutum*. Leiper also describes a large genital atrium, but as he says that he made his observations from a single sectioned specimen this cannot be regarded as of specific importance.

The only differences of importance therefore between the two species *C. sellsi* and *C. minutum* are those of size, and as quite as big a range of variation has been found in several series of *C. cotylophorum* examined by the writer, both with regard to the size of the whole worms and of the testes, it is considered probable that *C. minutum* and *C. sellsi* are identical.

Sub-family *CLADORCHINAE*, Fischoeder, 1901.

Definition.—Paramphistomidae : oral sucker with a pair of diverticula.

Key to Genera

1. Cirrus pouch absent ... ... *Pseudodiscus*
   Cirrus pouch present 2
2. Testes smooth ... ... *Balanorchis*
   Testes lobed or branched 3
3. Testes lobed ... ... *Pfenderius*
   Testes branched 4
4. Testes each consist of four branches like a cross ... ... *Chiorchis*
   Testes consist of many long irregular branches 5
5. Main portion of uterus posterior to testes, terminal portion runs ventral to testes ... ... *Taxorchis*
   Main portion of uterus arches dorsally over testes, terminal portion anterior to testes (as in *Paramphistomum*) ... ... *Cladorchis*

Genus *Watsonius*, Stiles and Goldberger, 1910.

Careful comparison of the definitions of *Watsonius* and *Pseudodiscus*, as given by Stiles and Goldberger, reveal little difference between the two.

In the definition of *Pseudodiscus* they state the genital pore is behind the gut fork; a ductus hermaphroditicus is present; the oral sucker is 'prominently constricted at equator'; the oesophagus has no muscular thickening; and the testes lie side by side near the middle of the worm and are 'cauliflower-like,' which means lobed with lobular subdivisions.

The same points in *Watsonius* are described as follows:—The genital pore is in front of the gut fork; a ductus hermaphroditicus is apparently absent; no mention is made as to whether the oral sucker is constricted or not; the oesophagus has a distal thickening of the muscle layer; and the testes are lobulate lying almost one behind the other. All the other points cited are the same in the two genera, or else are not of any value.

It is repeatedly shown in the present paper that the position of the genital pore in front or behind the gut fork is not even of specific value. As the constriction of the oral sucker is only alluded to in one genus, this point may be discarded. The differential diagnosis thus rests on the apparent absence of a ductus hermaphroditicus, the presence of an oesophageal thickening and the tandem arrangement of the testes in *Watsonius*, against the presence of a ductus hermaphroditicus, the absence of an oesophageal muscular thickening, and the lateral position of the testes in *Pseudodiscus*. In the sub-genus, *Hawkesius* of *Pseudodiscus*, however, Stiles and Goldberger state that there is a pronounced thickening of the posterior part of the oesophagus, and the testes are tandem. In respect of these two characters, the definition of *Pseudodiscus* as given by them cannot be correct. Thus, all the differential points between the genera *Pseudodiscus* and *Watsonius* are eliminated, except the doubtful absence of a ductus hermaphroditicus in the latter. The fact that Stiles and Goldberger in erecting the genus *Watsonius* had only a single specimen cut in transverse section, which they borrowed from Shipley, makes this point of very doubtful value, because it is so difficult to make out a ductus hermaphroditicus in sections of this nature, that it is unsafe to rely on them without confirmation from examination of sagittal sections. Leiper (1913), however, in a short record of *W. watsoni* figures the ductus ejaculatorius and uterus opening separately on the end of a prominent papilla, there being a narrow tongue-like process between the two; thus he apparently confirms

Stiles and Goldberger's statement that a ductus hermaphroditicus is absent. In the same paper, Leiper gives a drawing of *Gastrodiscoides hominis* in which the genital papilla and the two ducts are shown to be practically identical with his figure of *W. watsoni ;* the writer has examined sagittal sections of three specimens of *G. hominis* and found a papilla with a long ductus hermaphroditicus in the first specimen (fig. 29), a much shorter ductus hermaphroditicus in the second, and in the third there was no papilla at all and the male and female ducts opened separately at the bottom of a deep atrium (fig. 28). It seems probable that the same variation could be found if a number of *W. watsoni* were examined ; this is all the more likely when it is remembered that the same range of variations has been found in every species which the writer has been enabled to examine.

This discussion indicates that there are no clear differences between the genera *Pseudodiscus* and *Watsonius*, and that the latter should be merged in the former.

It should be noted that Railliet, Henry and Joyeux (1912), in recording *Watsonius watsoni* from *Cercopithecus callitrichus*, draw attention to the great similarity between this worm and *Hawkesius*, the sub-genus of *Pseudodiscus* created by Stiles and Goldberger (1910).

Genus *Pseudodiscus*.*

Synonymy :—*Watsonius*, Stiles and Goldberger, 1910.

Definition.—Cladorchinae : without a cirrus pouch, and testes lobed.

Type species *Pseudodiscus collinsi* (Cobbold, 1875), Sonsino, 1895.

Key to Species

Testes side by side	*P. collinsi*
Testes tandem	*P. hawkesii* and *P. watsoni*

Pseudodiscus collinsi (Cobbold, 1875), Sonsino, 1895.

Synonymy :—*Pseudodiscus stanleyi* (Cobbold, 1875), Sonsino, 1895.

First found in the colon of the horse in India.

The material investigated all came from India, and was as follows :—

* Stiles and Goldberger divide the genus *Pseudodiscus* into two sub-genera, viz., *Pseudodiscus*, and *Hawkesius*, but as this serves no useful purpose, it is not used in the present paper.

Bottle 1. Four specimens from a pony. These were all about the same size, viz., 7 mm. long and 5 mm. broad, and none contained eggs.

Bottle 2. Three specimens from a pony. Two of these were about 7·9 mm. long by 5·4 mm. broad, and both had eggs in their uteri. The third specimen was 5·7 mm. long by 4·4 mm. broad, and had no eggs.

Bottle 3. Three specimens from a horse. All three were very small, the largest being only 2·9 mm. long by 1·3 mm. broad.

Bottle 4. One specimen from a mule. About medium size.

Bottle 5. Three specimens from a horse. They were all small and averaged 4·3 mm. long by 2 mm. broad, and were all obviously immature.

Bottle 6. Sixty-four specimens from a horse. The maximum length of any of these worms was 4·8 mm. and the maximum breadth 2·5 mm., the minimum length was 3·2 mm. and the minimum breadth 1·3 mm., with all gradations between these two dimensions. But the longest worm was only 2·1 mm. broad, and the broadest worm was only 4·7 mm. long, therefore, the proportion of length to breadth is not absolute. The average of the whole sixty-four specimens was 4·4 mm. long by 1·9 mm. broad.

Bottle 7. Thirty-five specimens from a horse. These worms varied between 8·7 mm. long by 5·7 mm. broad and 5·4 mm. long by 3·2 mm. broad, and several of the larger specimens contained eggs.

(Bottles 6 and 7 were kindly lent by Mr. A. W. N. Pillers, F.R.C.V.S.).

For convenience, the points in which the two worms *P. stanleyi* and *P. collinsi* differ, according to Stiles and Goldberger (1910), are arranged in tabular form (Table VII).

Table VII.

	P. collinsi	*P. stanleyi*
1. Oesophageal portion of sucker.	Relatively broad. A dorsal and ventral 'transverse projecting ridge present.'	Relatively narrow. A dorsal ridge only present.
2. Testes.	Actually and relatively smaller.	Actually and relatively larger.
3. Opening of Laurer's canal.	A little above the posterior border of the acetabulum.	A little behind the anterior border of the acetabulum.
4. Size of acetabulum.	Relatively larger.	Relatively smaller.
5. Position of ovary and shell gland	About level of upper margin of acetabulum.	Distinctly further forward and nearer testes.

It must be noted, however, that Stiles and Goldberger record that they had only a few specimens of each of the two worms and, as they state that no eggs were seen, it is doubtful whether their worms were fully developed.

On looking through the Liverpool material, a doubt was raised in one's mind whether the differences described by Stiles and Goldberger were really of specific value, as it appears from our material that all intermediate stages between the two extremes were to be seen. With a view to examining this more fully, Table VIII was compiled from data obtained from Stiles and Goldberger (1910), from measurements of their drawings, and also from those of nine specimens taken from bottles 1, 2, 3, 6 and 7 of the Liverpool material, as far as possible representing all sizes of the worms.

Comparison of the dimensions given in the table makes it clear that Stiles and Goldberger's differences referring to the relative size of the oesophageal portion of the sucker, and the relative size of testes and acetabulum, are merely due to the stage of development of the various specimens that they examined. In all trematodes the digestive and fixation organs develop earlier than the sexual glands ; therefore, it is to be anticipated that the former are relatively larger and the latter relatively smaller in young worms than in older specimens. The differences of position of the ovaries and openings of Laurer's canal are very minute, and the above authors had so little material available, that to give specific value to slight variations in positions of these organs, as they have done, does not appear to be justified.

Historical. Cobbold (1875a) examined thirty-three specimens of this worm from a horse in India, which were sent him by Collins, and he named them *Amphistoma collinsi* because they were smaller than a similar fluke of the elephant (*A. hawkesii*) from the same locality. A few days after publication of this paper he received from Professor Simonds another bottle of flukes from the horse, collected by Stanley in India, and in a paper (Cobbold, 1875b) he named them *Amphistoma stanleyi*, but in doing so, says ' This is apparently nothing more than a large variety of the above ? ' He made no detailed study of the worms in either case, and no adequate description of the internal anatomy was published until Stiles and Goldberger (1910) examined some of Cobbold's original material. The number of individuals they had for study was not large, therefore they were led to making specific characters of differences which are really due to differences in age of the specimens they examined.

Pseudodiscus stanleyi (Cobb., 1875) is therefore only an immature form of *Pseudodiscus collinsi* (Cobb., 1875) and must be regarded as synonymous with the latter.

TABLE VIII

	Worm	Length	Breadth	Ratio of length to breadth	Ratio of diameter of oesophageal part of sucker to diameter of worm	Testes			Acetabulum			Distance of ovary and shell gland in front of posterior sucker	Condition of uterus
						Size	Ratio of size to distance between	Ratio of size to size of worm	Total diameter	Diameter of aperture	Ratio of diameter to length of worm		
From Stiles and Goldberger, 1910	*P. stanleyi* ...	8·6 to 9	5·5 to 5·6	1 : 1·82	...	...	...	...	1·7	1 to 1·25	1 : 5	...	No eggs.
	P. collinsi ...	5 to 5·76	3·5 to 4	1 : 1·46	...	...	...	...	1·58 by 1·1	0·5 by 0·7	1 : 3·9	...	No eggs.
	Drawing of *P. stanleyi* ...	...	...	1 : 1·54	1 : 12	...	1 : 0·4	1 : 4·8	...	...	1 : 4·5	...	
	Drawing of *P. collinsi* ...	...	...	1 : 1·27	1 : 7	...	1 : 2	1 : 7·0	...	...	1 : 4·1	...	
Author's material	Spec. 1 ...	2·9	1·4	1 : 2·2	1 : 5·4	0·059	1 : 6·3	1 : 23·6	0·572	0·416	1 : 5	0·05	Rudimentary.
	Spec. 2 ...	4·5	2·3	1 : 1·9	1 : 7·3	0·178	1 : 2·9	1 : 13	0·833	0·468	1 : 5·4	0·26	Rudimentary.
	Spec. 3 ...	5·36	3·23	1 : 1·6	1 : 10	0·468	1 : 1·3	1 : 7	1·145	0·781	1 : 4·7	0·078	Rudimentary.
	Spec. 4 ...	5·67	4·3	1 : 1·3	1 : 11	0·39	1 : 2·6	1 : 11	1·8	0·937	1 : 3·1	Touching	Partly developed.
	Spec. 5 ...	6·0	5·2	1 : 1·3	1 : 14·3	0·989	1 : 0·57	1 : 5·2	1·66	1·04	1 : 3·6	0·156	Uterus with eggs.
	Spec. 6 ...	6·7	4·16	1 : 1·3	1 : 13	0·572	1 : 1	1 : 7	1·56	0·885	1 : 4·3	0·260	Well developed; no eggs.
	Spec. 7 ...	7·76	5·36	1 : 1·26	1 : 14	1·04	1 : 0·5	1 : 7·5	1·822	1·09	1 : 4·4	0·208	Few eggs in posterior part.
	Spec. 8 ...	7·8	5·9	1 : 1·3	1 : 17·5	1·04	1 : 0·55	1 : 5·6	1·8	1·09	1 : 4·3	0·31	Well developed; no eggs.
	Spec. 9 ...	8·7	5·7	1 : 1·5	1 : 18·3	0·885	1 : 0·64	1 : 6·4	1·875	1·145	1 : 4·7	0·26	Well developed; no eggs.

Pseudodiscus hawkesii (Cobbold, 1875), Sonsino, 1895.

In a list of Trematodes from the Asiatic elephant, Railliet, Henry and Bauche (1914b) give the two following species of Amphistomes :—

(*a*) *Pseudodiscus hawkesi* (Cobbold, 1875).

Synonymy :—

Amphistoma hawkesii, Cobbold, 1875, non Piana and Stazzi, 1900.
Pseudodiscus hawkesi, Sonsino, 1895.

(*b*) *Watsonius ornatus* (Cobbold, 1882).

Synonymy :—

Amphistoma ornatum, Cobbold, 1882.
Pseudodiscus ornatus, Sonsino, 1895.
Amphistoma hawkesi, Piana and Stazzi, 1900.
Hawkesius hawkesi, Stiles and Goldberger, 1910.
Watsonius ornatus, Railliet and Henry, 1912.

Stiles and Goldberger (1910) state that their species *Pseudodiscus* (*Hawkesius*) *hawkesii* is a synonym of *Amphistoma hawkesii*, Cobbold, 1875. It therefore seems clear that *Pseudodiscus hawkesi* (Cobbold, 1875), and *Watsonius ornatus* (Cobbold, 1882) are the same. As Cobbold's original spelling was *hawkesii* and not *hawkesi* the correct name of the worm is *Pseudodiscus hawkesii* (Cobbold, 1875). This worm is easily distinguished from *P. collinsi*, because the testes in *P. hawkesii* are tandem and in *P. collinsi* they are placed side by side.

Pseudodiscus watsoni (Conyngham, 1904).

Synonymy :—

Amphistoma watsoni, Conyngham, 1904.
Cladorchis watsoni, Shipley, 1905.
Gastrodiscus watsoni, Verdun, 1907.
Paramphistomum watsoni, Manson, 1908.
Watsonius watsoni, Stiles and Goldberger, 1910.
Watsonius macaci, Kobayashi, 1915.

This species has been found on two occasions, once in man by Watson in 1904, and once in a monkey, *Cercopithecus callitrichus*, by Joyeux in 1912.

Comparison of Stiles and Goldberger's description of *P. hawkesii* and *P. watsoni* show that the two worms are practically identical. The size is somewhat different, however, *P. hawkesii* being 3·5 to 5 mm. in length by 2 mm. to 3 mm. in breadth, and *P. watsoni* 8 mm. to 10 mm. in length by 4 mm. to 5 mm. in breadth ; but in *P. hawkesii* no eggs were seen, whereas in the single specimen of *P. watsoni* which these observers examined eggs were present. The difference in size is therefore probably due to difference in age. The writer is of the opinion that the two species are identical, but in view of the facts that he has not been enabled to examine either, and that one worm comes from man and the other from the elephant, he does not feel justified in merging them.

Kobayashi (1915) recorded *Watsonius macaci* from *Macacus cynomolgus* and the same author (1920) gives a description of the worm, adding a footnote to the effect that it is probably identical with the fluke identified as *W. watsoni* by Railliet, Henry and Joyeux (1912). If this is correct, *W. macaci* is a synonym of *P. watsoni.*

Genus *Balanorchis*, Fischoeder, 1901.

Definition.—Cladorchinae : testes not lobed or branched ; cirrus sac present and protrusible ; genital sucker present.

Type species *Balanorchis anastrophus*, Fischoeder, 1901.

Host :—*Cervidae* sp. Location :—First stomach. Locality :—Brazil.

Only one species described.

Genus *Pfenderius*, Stiles and Goldberger, 1910.

Definition.—Cladorchinae : testes lobed ; cirrus sac present ; genital sucker absent.

Type species *Pfenderius papillatus* (Cobbold, 1882), Stiles and Goldberger, 1910.

Host :—*Elephas indicus.* Location :—Colon. Locality :—India.

Only one species described.

Genus *Chiorchis*, Fischoeder, 1901.

Definition.—Cladorchinae ; with testes each consisting of four branches arranged like a cross ; genital sucker absent and cirrus pouch present ; vitellaria in two narrow rows along outer side of caeca, each follicle group

has a duct joining direct with the main longitudinal duct; uterus as in *Paramphistomum*.

Type species *Chiorchis fabaceus* (Diesing, 1838), Fischoeder, 1901.

Host :—Marine mammals. Location :—small and large intestine. Locality :—?

Only one species described.

*Taxorchis** (Fischoeder, 1901), gen. nov.

Definition.—Cladorchinae : testes branched; cirrus pouch present; genital sucker present; main portion of uterus posterior to testes, terminal portion runs anteriorly ventral to testes.†

Type species *Taxorchis schistocotyle*, Fischoeder, 1901.

Host :—*Dictocotyle torquatus*. Location :—Caecum. Locality :—Brazil.

Only one species described.

Genus *Cladorchis*,‡ Fischoeder, 1901.

Definition.—Cladorchinae : with branched testes, genital sucker usually present; cirrus sac present; uterus as in the genus *Paramphistomum*.

Type species *Cladorchis pyriformis* (Diesing, 1838), Fischoeder, 1901.

Key to Species

Testes in tandem	1
Testes side by side	2
1. Posterior sucker on ventral surface at junction of posterior and middle thirds, small and contains no papillae ...	*C. pyriformis* (Diesing, 1838), Fischoeder, 1901. Host :—*Tapirus americanus*, S. America

* Fischoeder classified the species *T. schistocotyle* under the genus *Cladorchis*, sub-genus *Taxorchis*. But the course of the uterus is so different from any other member of the genus that it is considered advisable to place this species in a separate genus.

† The uterus arises from the shell gland just in front of the posterior sucker; from this point it runs forward near the dorsal surface to the posterior border of the testes, it then turns posteriorly and runs diagonally towards the ventral surface to just in front of the posterior sucker; after reaching this point it again turns forward and runs close to the ventral surface to the genital pore.

‡ Fischoeder divided this genus into three sub-genera, viz., *Cladorchis*, *Stichorchis*, and *Taxorchis*. The last has already been dealt with, having been raised to generic rank. *Cladorchis* and *Stichorchis* do not present striking anatomical differences, so they will be considered together, the sub-generic titles being dropped.

	Posterior sucker occupies hinder half of ventral surface ; large (2 mm.-4 mm.) and beset with papillae with special structure	*C. asper*, Fischoeder, 1901. Host :—*Tapirus americanus*, S. America
2.	Pharyngeal pouches projecting from sucker ; genital sucker distinct ...	*C. giganteus* (Diesing, 1838), Fischoeder, 1901. Host :—*Dicotyles* spp.
	Pharyngeal pouches so small that they do not show on external wall of sucker ; genital sucker not distinct	*C. subtriquetrus* (Rudolphi, 1814), Fischoeder, 1901. Host :—*Castor fiber*, *Bos taurus*.

Sub-family *STEPHANOPHARYNGINAE*, Stiles and Goldberger, 1910.

Definition.—Paramphistomidae : with a single oral diverticulum.
Only one genus.

Genus *Stephanopharynx*, Fischoeder, 1901.

Definition.—That of the sub-family.
Type species *Stephanopharynx compactus*, Fischoeder, 1901.
Only one species recorded.

Stephanopharynx compactus, Fischoeder, 1901.

Fischoeder found the worm in *Bos* sp.

The material available to the writer consisted of the following collections :—

1. About 850 specimens.
2. Over one hundred specimens.
3. Ten specimens.

These collections were found in the stomachs of three waterbuck (*Cobus* sp.) at Ngoa, N.E. Rhodesia.

Fischoeder's material consisted of two collections composed of a single specimen in one case and two specimens in the other ; they were found in company with many other *Amphistomata*.

In addition to the single large oral diverticulum, this worm is characterised by the anterior end being hemispherical instead of the usual conical type, and its maximum transverse diameter is about its middle instead of

towards the posterior end which is the ordinary condition in *Paramphistomidae*. The posterior sucker looks ventrally on account of the strong downward curve which the hinder half of the body exhibits, and it is said to have a surprisingly sharp border around its opening. The testes lie one behind the other, the anterior one being about the middle of the body, with the hinder one between it and the posterior sucker. Laurer's canal opens in front of the excretory pore in the mid-line of the dorsal surface, and the genital pore is surrounded by a thickening of the subcuticular muscle not sharply marked off from the parenchyma. The size of these worms is given as 4·8 to 5 mm. in length, 3 mm. in breadth, and 2·5 mm. in thickness. Other anatomical details are of the usual type. The writer found in his collection No. 1 eight worms, and in his collection No. 2 one worm which agreed with Fischoeder's description in all particulars, except that they were about 7 mm. in length. The remaining numerous specimens, however, at first sight appeared to be very different, as they were sharply pointed at the anterior end and the posterior sucker looked directly backwards, in many cases being widely opened and occupying the whole hinder end of the worm. (Plate VII, fig. B). These worms were of all sizes from tiny specimens about 2 mm. in length up to worms 5·5 mm. in length. Several of these worms were sectioned and it was then found that they agreed with Fischoeder's species, except that the posterior sucker was relatively larger, and the hinder of the two testes was much nearer the ventral surface than the anterior one (fig. 12). At first it was thought that these points indicated a new species, but it was then noted that none of the worms were gravid, and prolonged search of the ample material failed to reveal any specimen containing eggs. On this account it is considered that they are immature specimens of *S. compactus*, which fact explains the relatively large size of the posterior sucker. The apparently different position of the testes in young worms is not without parallel, for Fischoeder says that in immature *P. calicophorum*, the anterior testis lies more dorsal than the hinder one, a character which the writer has found disappears when maturity is reached. A further fact in support of the view that the present worms are only immature *S. compactus* is that this species has only been recorded on five occasions (twice by Fischoeder and three times by the writer) and on two of these it has been found in company with worms of the above closely allied type.

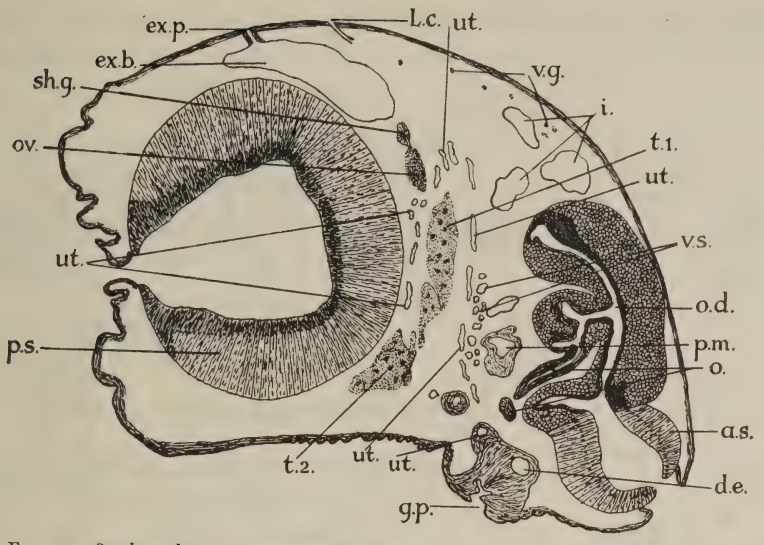

Fig. 12. *Stephanopharynx compactus.* Sagittal section near the mid-line. *a.s.*—anterior sucker; *d.e.*—ductus ejaculatorius; *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *g.p.*—genital pore; *i.*—intestine; *L.c.*—Laurer's canal; *o.*—oesophagus; *o.d.*—oral diverticulum; *ov.*—ovary; *p.m.*—pars musculosa; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t1.*—dorsal testis; *t2.*—ventral testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.s.*—vesicula seminalis. × 14.

Family *GASTROTHYLACIDAE*, Stiles and Goldberger, 1910.

Definition.—Amphistomata : with a ventral pouch.

Key to Genera

1. Uterus crosses from one side of body to the other near the middle of the worm ... ... *Gastrothylax*
   Uterus lies in centre of body for its whole length 2
2. Testes side by side ... ... *Carmyerius*
   One testis dorsal of the other, both in mid-line ... ... *Fischoederius*

Genus *Gastrothylax*, Poirier, 1883.

Fischoeder (1903) placed all the known *Paramphistomidae* with a ventral pouch in the genus *Gastrothylax*. He divided the species in this genus into five groups, using the shape of the ventral pouch on cross section as the distinguishing feature. His list is as follows :—

Genus *Gastrothylax*.

(*a*) Transverse section of pouch triangular with apex dorsally directed and apical angle undivided.

1. *G. crumenifer* (Crepl.).
2. *G. compressus*, Brandes.

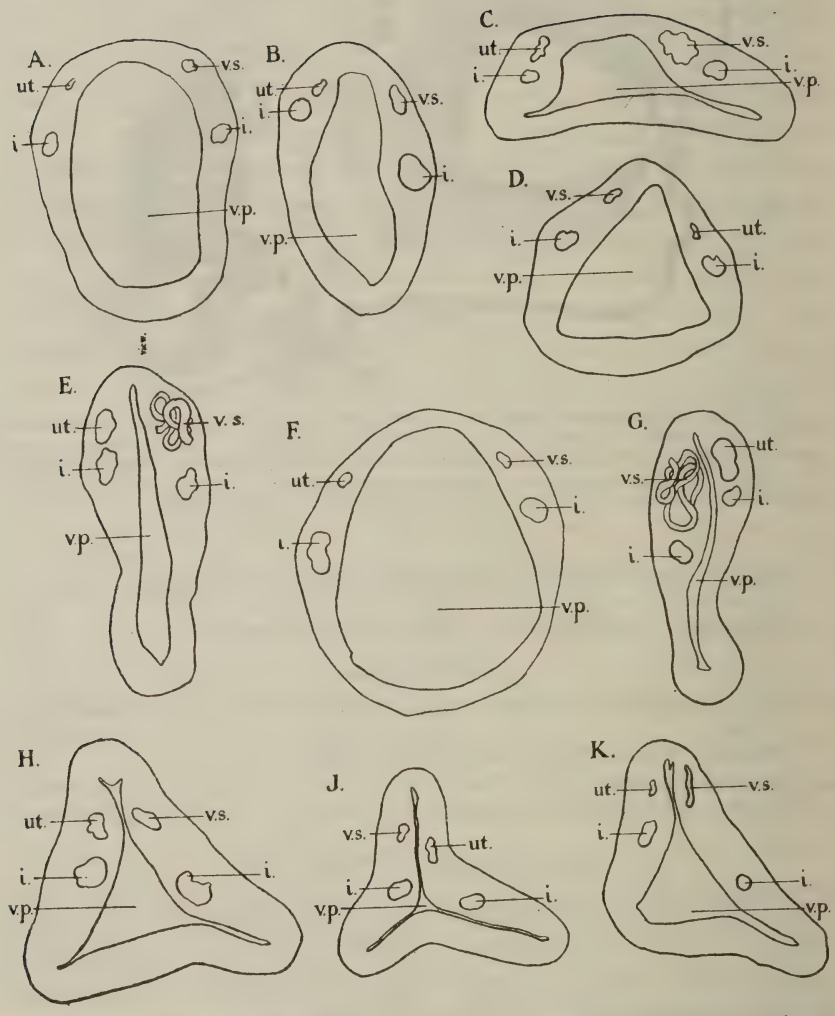

FIG. 13. *Gastrothylax crumenifer*. Transverse sections through the middle of ten specimens showing variation in shape of ventral pouch. *i.*—intestine; *ut.*—uterus; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis. × 9.

(*b*) Transverse section of pouch triangular with apex dorsally directed and apical angle bifurcated.

3. *G. gregarius*, Looss.

(*c*) Transverse section of pouch circular.

4. *G. spatiosus*, Brandes.

(*d*) Transverse section of pouch triangular with apex ventrally directed.

5. *G. synethes*, Fischdr.
6. *G. elongatus*, Poirier.
7. *G. cobboldi*, Poirier.
8. *G. mancupatus*, Fischdr.

(*e*) Transverse section of pouch triangular with apex ventrally directed. The two basal angles bifurcated.

9. *G. minutus*, Fischdr.

The writer has carefully investigated the shape of the ventral pouch in cross section in all the species he has had at his disposal. Fig. 13 illustrates transverse sections at the mid point of ten specimens of *G. crumenifer*. All the specimens agreed in other anatomical details and all came from the same bottle. Fig. 14 represents similar sections of six specimens of *G. spatiosus*.

On examining the drawings it will be seen that fig. 13, D, corresponds with Fischoeder's group (*a*), fig. 13, H and K, correspond with his group (*b*), and fig. 13, F, is so nearly circular as to correspond with his group (*e*). Fig. 13, A, B, C, E and J, do not agree with any of Fischoeder's five groups. Therefore, in a series of ten specimens of a single species three of Fischoeder's five groups are represented, and the others cannot be classified by this method. If fig. 13, D, H and K, are further examined, it will be noted that they are all triangular with the apex dorsal, whereas in fig. 14, B and D, which approach the triangular shape, the apex is ventral. This at first seems to indicate that a division between the species might be made on the fact that when the pouch is triangular, the apex is ventral in some species and dorsal in others. But fig. 14, A, upsets this view, because here is a worm considerably distorted in fixation with the dorsal surface drawn to the right and the ventral surface displaced to the left. The ventral pouch in this case is a narrow triangle with the base to the right. If this worm could be straightened up, one would have a specimen with the base

of the ventral pouch looking towards the ventral surface, unlike the other examples of the same species in which the base of the pouch, when triangular, is dorsally directed. From this it will be seen that the cross section of the ventral pouch may assume almost any shape and cannot therefore be regarded as of any use in diagnosis.

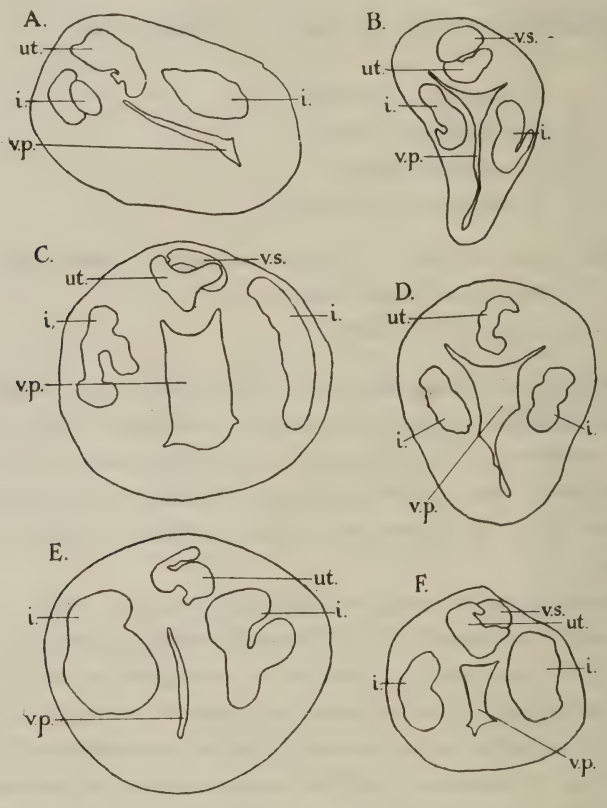

FIG. 14. *Carmyerius spatiosus.* Transverse sections through the middle of six specimens showing variations in shape of ventral pouch. *i.*—intestine; *ut.*—uterus; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis. × 9.

The shape of the ventral pouch when seen from the side also varies considerably, as reference to figs. 15, 16, and 17 will readily show. For the most part the changes in shape of the pouch when viewed in this position are caused by outpocketings from the posterior end along the dorsal and ventral surfaces of the testes, but in a few cases a narrow prolongation may also occur from the dorso-anterior part of the pouch (see fig. 17, C). In addition to differing as a whole, the pouch also exhibits varying form in

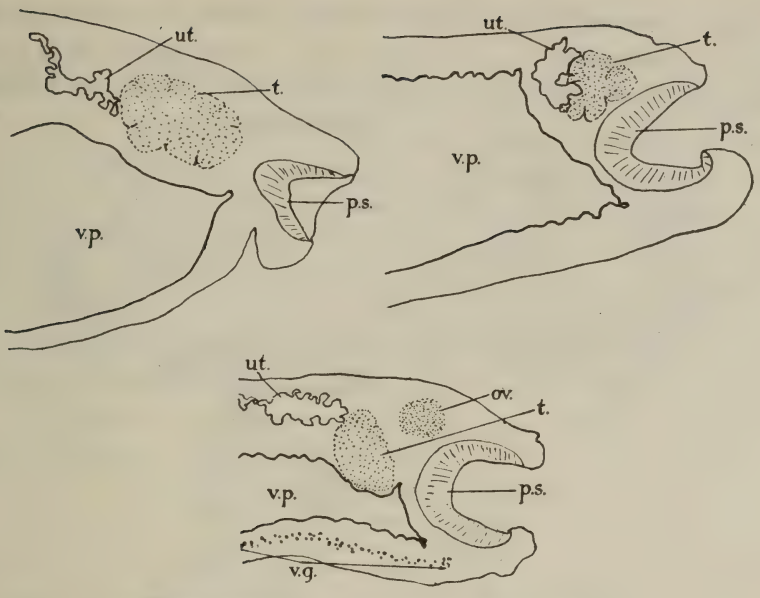

FIG. 15. *Gastrothylax crumenifer.* Sagittal sections near the mid-line of three specimens to show the shape of the posterior end of the ventral pouch. *ov.*—ovary; *p.s.*—posterior sucker; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch. × 9.

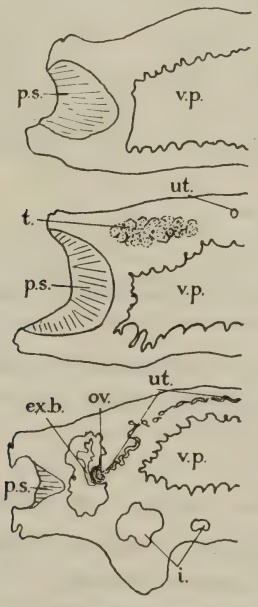

FIG. 16. *Gastrothylax crumenifer.* Three sagittal sections from a single specimen at different levels, showing change in shape of posterior end of pouch in one worm. *ex.b.*—excretory bladder; *i.*—intestine; *ov.*—ovary; *p.s.*—posterior sucker; *ut.*—uterus; *v.p.*—ventral pouch. × 9.

different sections of the same worm (fig. 16). It will be noted later that Stiles and Goldberger use the presence of a ventral prolongation of the pouch beneath the testes as a specific character in at least one instance.

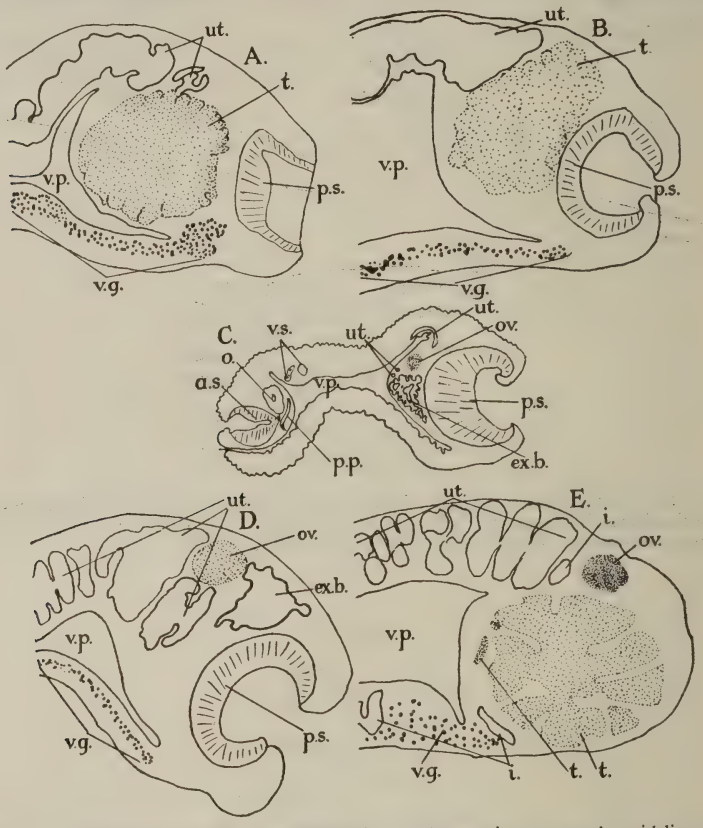

Fig. 17. *Carmyerius spatiosus.* Sagittal sections of five specimens near the mid-line showing variation in shape of posterior end of pouch. *A*, *B*, *D*, and *E*—Gravid worms. *C*—Immature worm. *a.s.*—anterior sucker; *ex.b.*—excretory bladder; *i.*—intestine; *o.*—oesophagus; *ov.*—ovary; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis. × 9.

The above demonstration of the presence or absence of this prolongation in a single species (fig. 17) is held to prove that this character is of no value for specific diagnosis.

Genus *Gastrothylax* (Poirier, 1883), s. str., Stiles and Goldberger, 1910.

Definition.—Gastrothylacidae: with the uterus crossing from one side of the body to the other about its middle.

Type species *Gastrothylax crumenifer* (Creplin, 1847).

Only one species recorded.

Gastrothylax crumenifer (Creplin, 1847).

Synonymy :—*Gastrothylax compressus*, Brandes, 1898.

Stiles and Goldberger (1910) revised Fischoeder's classification and restricted the genus *Gastrothylax* to include only those species in which the uterus crosses from one side of the worm to the other about midway between the anterior and posterior extremities. The result of this restriction is that the genus now contains only two species, viz., *G. crumenifer* and *G. compressus*. The writer has not been able to consult the original descriptions of these species, but Fischoeder (1903) describes them very fully and use has been made of this work in the present discussion.

The points of difference between these two worms are summarised from Fischoeder (1903) in Table IX.

Table IX.

Points of difference between *G. compressus* and *G. crumenifer*.

	G. compressus	*G. crumenifer*
Length of oesophagus ...	400μ to 500μ	1·2 mm. to 1·5 mm.
Gut caeca	Almost straight. End 300μ to 500μ anterior to testes.	Wavy. End behind anterior border of testes.
Uterus	Not so convoluted.	More convoluted.
Eggs	Not so numerous. 115μ to 125μ by 60μ to 65μ.	More numerous. 125μ to 135μ by 65μ to 70μ.

In the present instance the material available for examination consisted of two bottles containing several hundred specimens. One collection came from a bullock in India and the other from a bullock in Hong Kong. Examination of a large number of these worms has shown that like all other *Amphistomata*, they are liable to considerable variation. In our collections there are many worms which could be identified as one or other of the above species, and there are just as many which would fit either species just as well. It is therefore considered that these two species have been separated from one another on points of individual variation, and that the differences are so variable in degree that in all probability *G. compressus* is merely a synonym of *G. crumenifer*.

Genus *Fischoederius*, Stiles and Goldberger, 1910.

Definition.—Gastrothylacidae : with the uterus in the centre of the body for its whole length ; one testis dorsal of the other.

Type species *Fischoederius elongatus* (Poirier, 1883), Stiles and Goldberger, 1910.

Key to Species

Gut caeca extend at least to testes	*F. cobboldi*
Gut caeca never extend further than just beyond middle of body	*F. elongatus*

Fischoederius elongatus (Poirier, 1883), Stiles and Goldberger, 1910.

Synonymy :—

Fischoederius fischoederi, Stiles and Goldberger, 1910.
Fischoederius siamensis, Stiles and Goldberger, 1910.
Fischoederius ceylonensis, Stiles and Goldberger, 1910.

First found in the stomach of *Palonia frontalis* in Java.

The material available to the writer consisted of about 25 specimens obtained from a bullock in Hong Kong.

Eight specimens were cleared in carbolic acid and their examination showed that the worm closely agrees with Fischoeder's (1903) description, with the proviso that this author does not take cognisance of individual variations. For instance, Fischoeder states that the caeca end within the beginning of the hinder half of the body, but in the eight specimens examined, this was found to be the case only in three of them ; in one the caeca ended exactly at the junction of the anterior and posterior halves of the body, and in the remaining four specimens they ended at varying distances up to as much as 1 mm. in front of it. In three cases the two caeca were of unequal length ; in the most marked instance the gut on one side was 1 mm. longer than on the other. In some of the worms examined, the caeca were as convoluted as Fischoeder shows in his fig. 59, but in others they were nearly straight, and furthermore some caeca presented short dilated portions in various parts of their course, while in others they were of uniform diameter throughout.

Fischoeder also says that the testes lie one above the other near the mid-line, the ventral one being slightly to the right of the mid-line and slightly posterior to the dorsal, which is rather to the left of the mid-line. This was true of some of the specimens examined, but in others one testis appeared to lie directly above the other, and both of them were exactly in the mid-line.

Fischoederius fischoederi, Stiles and Goldberger, 1910.

This species is stated to have been made from a single specimen found in a bottle containing a number of worms which had been determined by Fischoeder as *G. elongatus* (*F. elongatus*). They distinguished it from the latter on the ground that the gut caeca were very slightly longer, and that the ovary and shell gland lie between the testes in *F. fischoederi*, which they state is not the case in *F. elongatus*. With regard to the position of the ovary in *F. elongatus*, Fischoeder (1903) states:

'Der fast kuglige Keimstock (0·3 bis 0·35 mm. im Durchmesser) liegt dorsal von der Schalendrüse, etwas seitlich von der Medianlinie dicht hinter und etwas median von dem meist nach hinten herabhangenden dorsalen Ende des vordern Hodens (Text fig. L).'

It will be noted that he makes no reference to the other testis, but Text fig. L, to which he refers, shows the ovary and shell gland lying between the two testes, but a little further back than Stiles and Goldberger show them in fig. 2. As it is recognised that the ovary varies slightly in position in most species, this difference cannot be considered of specific importance, especially as it relates to an isolated specimen taken from a collection of *F. elongatus* which had already been determined by Fischoeder. It is therefore considered that *F. fischoederi* is a synonym of *F. elongatus*.

Fischoederius siamensis and *F. ceylonensis*, the other two new species made by Stiles and Goldberger, are separated from *F. elongatus* because they do not exhibit the prominent bulging round the genital pore which these authors state is present in *F. elongatus*. It is true Fischoeder (1903) in his description of *F. elongatus* mentions this bulging, but from the experience of the writer, it is concluded that the bulging is a variable character and is similar to that which may be found in any species of the group *Amphistomata* and is accordingly of no specific value. It is therefore considered that *F. siamensis* is synonymous with *F. elongatus*.

F. ceylonensis was established as a new species on a single specimen taken from a bottle of *G. synethes*. Stiles and Goldberger distinguish between *F. ceylonensis* and *F. siamensis* by two points; firstly, the testes in *F. ceylonensis* are directly one above the other and in *F. siamensis* they are slightly diagonal; and secondly, the ventral pouch in *F. ceylonensis* extends ventral to the testes, whereas in *F. siamensis* this extension of the ventral pouch does not occur. The individual variations which may be found in the ventral pouch have, however, already been fully discussed,

and it is considered that the differences between *F. ceylonensis* and *F. siamensis* are not of specific value, more especially as the diagnosis of one of them rests on a single specimen.

Fischoederius cobboldi (Poirier, 1883), Stiles and Goldberger, 1910.

First found in the stomach of *Palonia frontalis* in Java.

No material of this species was available to the writer.

From Fischoeder's description, the distinguishing feature between *F. cobboldi* and *F. elongatus* is the difference in position of the termination of the caeca. In *F. cobboldi* they end opposite the base of the sucker, that is posterior to the testes and near the hinder end of the worm, and in *F. elongatus* they end about the middle of the worm. Although it has been shown that the point of termination of the caeca is subject to variations in practically all species of the group, in the present instance the differences between the two species under discussion are so marked that it is unlikely that they can be explained in this manner. It is accordingly considered that *F. cobboldi* and *F. elongatus* can be distinguished by the difference in length of the caeca.

Genus *Carmyerius*, Stiles and Goldberger, 1910.

Synonymy :—*Wellmanius*, Stiles and Goldberger, 1910.

Definition.—Gastrothylacidae : with the uterus in the centre of the worm for its whole length ; testes side by side.

Type species *Carmyerius gregarius* (Looss, 1896), Stiles and Goldberger, 1910.

Key to Species

1.	Genital pore lies outside ventral pouch	*C. exoporus*
	Genital pore lies within ventral pouch	2
2.	Excretory canal and Laurer's canal unite before reaching the surface	*C. wenyoni*
	Excretory canal and Laurer's canal do not unite	3
3.	Cross section of ventral pouch shows five angles	*C. cruciformis*
	Cross section of ventral pouch does not show five angles	4
4.	Gut caeca extend to testes	*C. spatiosus*
	Gut caeca do not extend beyond middle of worm	*C. gregarius*

Stiles and Goldberger made this genus to include all the species of Poirier's *Gastrothylax* in which the uterus occupies a central position for its whole length, the testes lie side by side, and the vas deferens is without a straight portion at its commencement. This included the following five

species : *C. synethes* (Fischoeder, 1901), *C. gregarius* (Looss, 1896), *C. spatiosus* (Brandes, 1898), *C. mancupatus* (Fischoeder, 1901), and *C. minutus* (Fischoeder, 1901). They divide the above five species into two groups, because the first two worms are said to have a genital atrium with a large ventral chamber and the last three a genital atrium without a ventral chamber. These characters have been fully discussed already and it has been shown that presence or absence of chambers in the atrium, or even the existence of the atrium itself, are purely matters of chance, so that it seems reasonable to assume that this point is of no value in the present instance. Differences in shape of the ventral pouch are also mentioned in the definitions of the various species. The unreliability of this character has already been dealt with.

Carmyerius spatiosus (Brandes, 1898).

Synonymy :—

Carmyerius synethes, Fischoeder, 1901.
Carmyerius minutus, Fischoeder, 1901.
Carmyerius mancupatus, Fischoeder, 1901.
Gastrothylax bubalis, Innes, 1912.
Wellmanius wellmani, Stiles and Goldberger, 1910.

First found in the stomach of *Bos taurus* in Arabia.

Material available :—Two collections from Ngoa, N.E. Rhodesia ; the host in one case was a roan (*Hippotragus equinus*) and in the other a reedbuck (*Cervicapra* sp.).

As the four species *C. spatiosus, C. synethes, C. minutus* and *C. mancupatus* only differ in minute points apart from those already dealt with, they will be discussed together.

According to Stiles and Goldberger's key, the only point by which these four species may be distinguished are minute differences in the gut caeca. They write that in *C. synethes* the caeca are ' corkscrew-like, rather narrow and long ' ; in *C. spatiosus* they are ' straight, narrow and rather long ' ; in *C. mancupatus* they are ' rather sinuous, narrow, and long ' ; and in *C. minutum* they are ' swollen in their caudal half and are rather long.' In all four cases the caeca are said to end in the ' fourth zone.' Twenty specimens were cleared in carbolic acid and it was found that the degree of convolution of the caeca was so variable as to be of no use for diagnosis. With regard to differences in diameter of various parts of the caeca, they were found swollen or contracted in any part of their course or of uniform

diameter throughout. Fig. 18, A and B are drawings of the two caeca in a single worm showing alternate contractions and dilatations ; fig. 18, C, is a drawing from another specimen showing the gut of uniform diameter from end to end. It is therefore clear that dilatation of a special part of the caeca is of no diagnostic value.

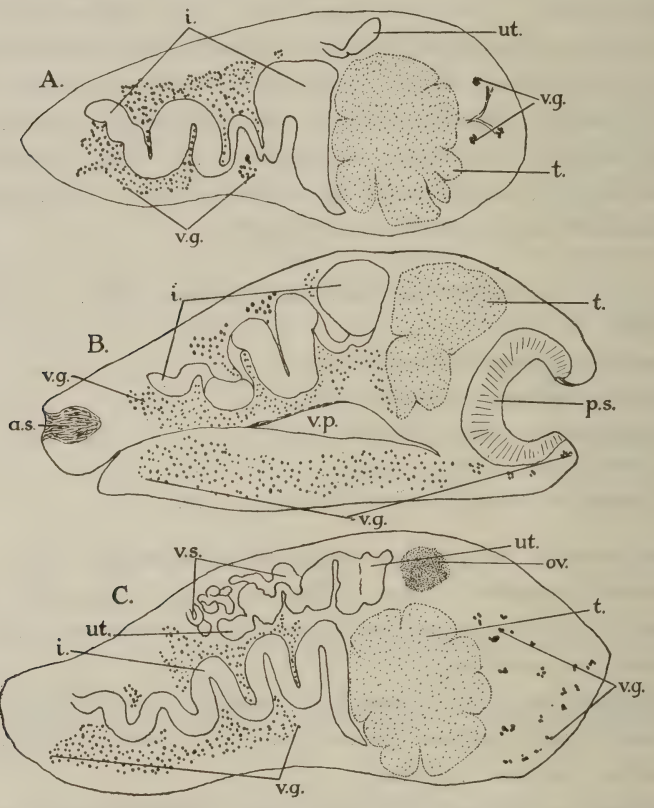

Fig. 18. *Carmyerius spatiosus.* Sagittal section to show variation in calibre of gut. *A* and *B*—Showing the two sides of the same worm. *C*—Section of another worm. *a.s.*—anterior sucker ; *i.*—intestine ; *ov.*—ovary ; *p.s.*—posterior sucker ; *t.*—testes ; *ut.*—uterus ; *v.g.*—vitelline gland ; *v.p.*—ventral pouch ; *v.s.*—vesicula seminalis. × 9.

Fischoeder (1903) mentions one or two other small points of difference, viz., the genital pore lies further posteriorly in *C. synethes* than in any other species, being about 1 mm. behind the entrance to the ventral pouch ; but as he gives 0·8 mm.-0·9 mm. and 0·7 mm.-0·8 mm. for the same dimension in *C. spatiosus* and *C. mancupatus* respectively, it cannot be considered justifiable to regard this of specific value, especially as he gives the limits of variation in length of the worms as being between 6 mm.-12 mm. for the three species.

Fischoeder states that *C. minutus* is 4 mm.-5 mm. in length ; then he remarks that some specimens are longer and thinner than this, but gives no other measurements. This point cannot be regarded of any value when he says that *C. spatiosus, C. synethes* and *C. mancupatus* are 9 mm.-12 mm., 7 mm.-11 mm., and 6 mm.-11 mm. in length respectively. Another point of difference claimed between *C. minutus* and the other three species, is that the ovary and shell gland are said to be more strongly developed in the former. But difference in size of the genital organs is so dependent on the age of an individual that it cannot be regarded as a satisfactory diagnostic character.

From the above observations it is concluded that Fischoeder's three species, *C. synethes, C. mancupatus* and *C. minutus* are synonyms of *C. spatiosus* (Brandes).

Carmyerius wenyoni (Leiper, 1908).

Found in the stomach of *Cobus maria* at Taufikia, White Nile.

It is not included in Stiles and Goldberger's (1910) classification. It is similar in most respects to *C. spatiosus*, but there is one point by which it may be clearly distinguished, and that is that the excretory canal and Laurer's canal unite before reaching the surface of the worm and open by a common pore. This character is very remarkable as it has not been observed in any other species.

Carmyerius cruciformis (Leiper, 1910).

The material available for study consisted of a single collection of over one hundred specimens from a hippopotamus killed in Lake Victoria, Nyanza.

This worm was first described by Leiper who states that he only had immature material. Leiper gives the length of this worm as from 0.5 mm. to 0·8 mm. ; this is apparently a mistake for 5 mm. to 8 mm. because he gives the dorso-ventral diameter of the posterior sucker as 0·9 mm. with a muscle wall of 1·8 mm. in thickness. Some of the writer's specimens contained eggs in the uterus and these worms measured 5 mm. to 7 mm. in length with a maximum diameter of about 1·5 mm. ; they agree in general anatomical details with Leiper's description. The uterus

was observed to pursue a slightly wavy course along the centre of the dorsal surface; the most heavily gravid specimen only contained about thirty eggs disposed in a single chain and not closely packed into the uterus in the usual manner. Eggs removed from one specimen measured about 135-140μ in length by 80-84μ in breadth; on account of the small size of the worm they appeared relatively large. Leiper named this worm 'cruciformis' because he says the ventral pouch always shows five angles in cross section no matter how contracted the worm may be, and this frequently appears as a cross. The writer cut eight specimens transversely, and although the pouch of three of them exhibited five angles none were at all like a cross and the other five specimens exhibited just as extensive variation as do all *Gastrothylacidae* in respect of the shape of the pouch.

This worm is essentially the same as *C. spatiosus* in details of anatomy, but is easily distinguished from this species by its minute size even in the gravid state.

Gastrothylax bubalis, Innes, 1912.

This worm was recorded by Innes (1912) as a new species. It was found in the stomach of the Hartebeest in Rhodesia.

The general anatomy of the worm is apparently in no way different from *C. spatiosus;* to quote from Innes the identification of the species is based on the following characters:—

'The excretory vesicle of this species is unique both in position and outline. It is situated immediately in front of the posterior sucker and behind the shell gland, while in other species it has a more or less lateral position. The great irregularity of its outline is striking.'

No authority is given for the statement that the excretory bladder lies in a more or less lateral position in other species, and Fischoeder (1903) states that in *G. spatiosus*:

'Die sehr grosse Excretionsblase liegt zwischen der Bauchtasche und dem Saugnapfe einerseits und den beiden Hoden andrerseits (fig. 52c. u. 54).'

And in all other species of the genus that he deals with he describes it in a similar position, only alluding to slight alterations due to change of shape, and his figures all show it in the same position as it is seen, Innes' fig. 7. It therefore seems clear that this difference claimed by Innes does not exist. With regard to his other point, viz., the irregularity of the excretory bladder, it may be observed that irregularity of outline is

characteristic of a partly distended bladder in any species, and is obviously of no specific value. From this it is concluded that the species *G. bubalis* is only a synonym of *C. spatiosus*.

Carmyerius gregarius (Looss, 1896), Stiles and Goldberger, 1910.

First found in the stomach of *Bos bubalis* in Egypt.

Material available.—A single collection of about 25 specimens from a buffalo.

This species is easily distinguished from the other species of the genus, as the gut caeca are very short. Of ten specimens cleared in carbolic acid only one was found in which the caeca reached the middle of the worm; in all the others they ended at varying distances anterior to this point. For the most part the caeca were of uniform diameter throughout, but in one specimen they exhibited a swelling about their middle and in another there was a club-shaped swelling at their extremities. Although variation in the length of the caeca has been found unreliable for specific diagnosis when the range is slight, they are, however, so much shorter in *C. gregarius* than in *C. spatiosus* that this difference is considered of specific value in the present instance.

Carmyerius exoporus, n.sp.

Found in the stomach of a *Tragelaphus spekei* in Nyasaland.

The material available was a single collection of over three hundred specimens.

External Anatomy

All stages of growth were represented in the collection, from worms no bigger than a raspberry seed up to gravid worms measuring over 11 mm. in length. But even in gravid worms the shape and size was subject to great variation (see Plate VII, A). It will be noted in this plate that in some cases the worms are fully extended (9) and in others they are in a state of contraction (7). In view of the great variation in shape that exists, it is not considered worth while giving a detailed description, but as a rough indication of what the size of gravid worms is, it may be stated that they varied from 11·5 mm. in length by 2·6 mm. in breadth to 5 mm. in length by 3·8 mm. in breadth. In all cases the worms were practically circular on cross section. As the worms are not curved ventrally, the opening of the anterior sucker lies at the extreme anterior end and looks directly

forwards. The opening of the ventral pouch lies in the mid-line of the ventral surface close behind and below the oral opening, and between the two is the opening of the genital pore, which is thus outside the ventral pouch. This character is sufficient to distinguish the present species from any other member of the genus *Carmyerius*, as in all the hitherto described species the genital pore opens within the ventral pouch. The posterior sucker, as a rule, looks directly backwards, but in a few cases it is slightly tilted ventrally.

Internal Anatomy

The general arrangement of the organs of this species is very similar to *G. spatiosus* and on that account only the briefest description is necessary.

Muscular system. This system exhibits no special characters.

Nervous system. This system was not investigated.

Anterior sucker. The anterior sucker is of the typical globular shape, oval in section.

Intestines. The oesophagus arises from the posterior end of the anterior sucker and after pursuing a dorsally curving course of about 300μ, it divides into the two gut caeca. These two canals pursue a wavy course along each side of the worm and terminate about the level of the testes.

Posterior sucker. The posterior sucker is fairly thick-walled and looks directly backwards or slightly towards the ventral surface (figs. 20, 21 A, and 25).

Excretory system. The excretory bladder is of the usual type and its degree of convolution varies with its state of distension. The excretory canal runs dorso-posteriorly and opens in the mid-line of the dorsal surface above the anterior border of the posterior sucker (fig. 20).

Genitalia. Testes. The testes are large lobed organs lying one on each side of the mid-line near the hinder end of the worm (fig. 21 A).

Vas deferens. This duct is composed of the usual three portions, the vesicula seminalis, the pars musculosa, and the pars prostatica. The first two portions are much convoluted and occupy the anterior third of the centre of the dorsal part of the worm. The pars prostatica is relatively long and straight, and runs directly forwards to enter the genital papilla (figs. 19 and 21, B).

Genital pore. The genital pore is surrounded by a muscular thickening not marked off from the parenchyma and it opens in the middle of the worm between the oral opening dorsally and the opening of the ventral pouch

ventrally (figs. 19 and 22). It is of the usual type in structure and the appearance shown in the figures is not the only one met with, as the genital papilla is capable of complete retraction or extrusion as in other species. In the figures it is in the intermediate condition.

Ovary and shell gland. These two structures lie between the testes on one hand, the base of the ventral pouch in front and the posterior sucker on the other (figs. 20 and 21 A).

Laurer's canal. Laurer's canal runs almost directly dorsally from the shell gland and opens in the mid-line well in front of the excretory pore (fig. 20).

Vitellaria. For the most part these glands lie in the ventral portion of the worm, but they extend for varying distances on each side towards the dorsal surface. As a rule, they extend from the posterior end of the anterior sucker in front, to opposite the testes behind, but in a few cases follicles were found close to the posterior end of the worm, and surrounding the opening of the posterior sucker (figs. 19, 20, 21, A, and 25).

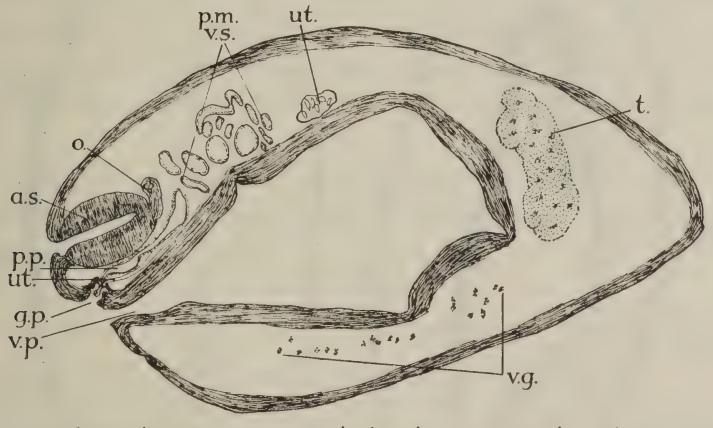

FIG. 19. *Carmyerius exoporus*, n.sp. Sagittal section. *a.s.*—anterior sucker; *g.p.*—genital pore; *o.*—oesophagus; *p.m.*—pars musculosa; *p.p.*—pars prostatica; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch. ×18.

Uterus. The uterus pursues a wavy course along the centre of the dorsal surface; anteriorly it terminates by running ventral to the pars prostatica and uniting in the genital papilla with the male duct (figs. 21 B and 22).

Eggs. These are oval, operculated and measure about 115μ to 130μ in length by 60μ to 68μ in breadth, but they were only taken from one specimen, so these dimensions must be regarded as only approximate.

Ventral pouch. The ventral pouch is somewhat characteristic in

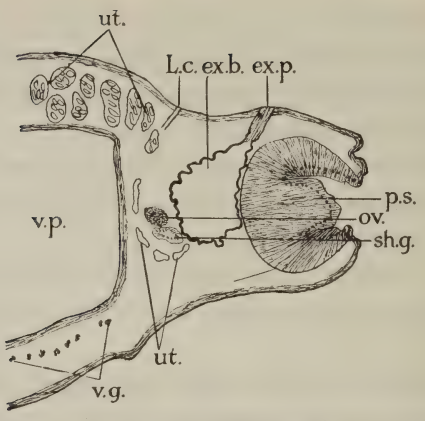

FIG. 20. *Carmyerius exoporus*, n.sp. Sagittal section of posterior end of worm. *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *L.c.*—Laurer's canal; *ov.*—ovary; *p.s.*—posterior sucker; *sh.g.*—shell gland; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch. × 16.

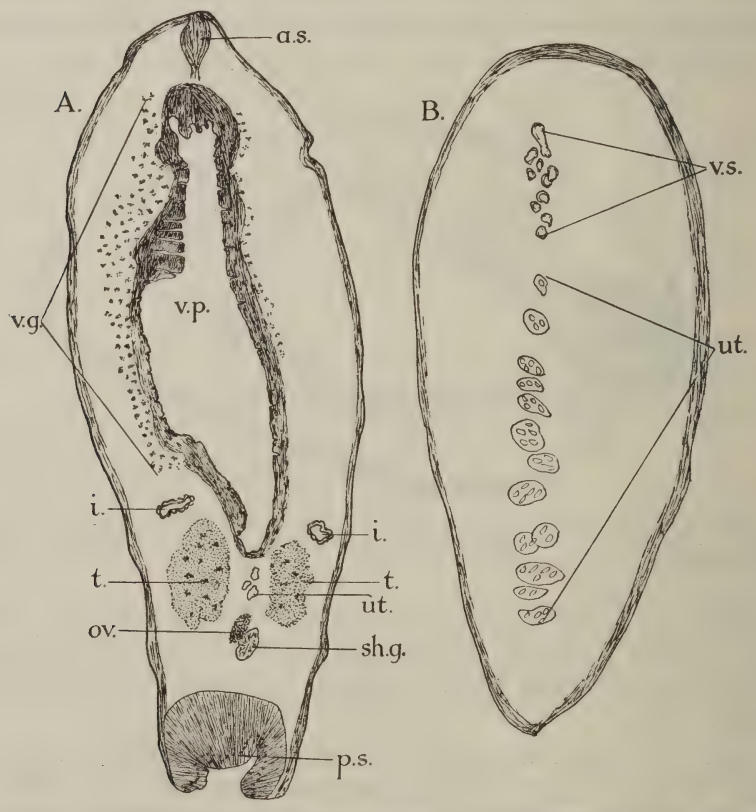

FIG. 21. *Carmyerius exoporus.* Coronal sections. *A*—About middle of worm. *B*—Near dorsal surface. *a.s.*—anterior sucker; *i.*—intestine; *ov.*—ovary; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t.*—testes; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis. × 8.

some cases, in that on cross section about its middle it often shows six points or angles ; but it is subject to so many variations that this cannot be regarded as a diagnostic character. These variations are well shown in figs. 23, 24 and 25.

Diagnosis

As this worm is easily distinguished by the fact that the genital pore opens outside the ventral pouch, the name *Carmyerius exoporus*, n.sp., is suggested.

Genus *Wellmanius*, Stiles and Goldberger, 1910.

This genus was established by Stiles and Goldberger to include a new species *Wellmanius wellmani* which was obtained from the stomach of a reed bok (*Cervicapra bohor*) at Benguella, West Africa. The sole distinguishing character between *Wellmanius* and *Carmyerius* is that in the former the first part of the vesicula seminalis is straight, and in the latter it is coiled from the commencement. This seems a very small point on which to establish a genus, especially when it is found that the beginning of the vesicula seminalis cannot be made out with accuracy in most gravid specimens whatever the species may be, because it is overlaid by the uterus. *W. wellmani* appears to the writer to be synonymous with *C. spatiosus*.

Family *GASTRODISCIDAE*, Stiles and Goldberger, 1910.

Definition.—Amphistomata : body usually flattened and divided into anterior and posterior portions.

Key to Genera

1. Anterior portion large and flat, and posterior portion smaller and spherical *Homalogaster*
   Anterior portion small and conical, posterior portion large and flat ... 2
2. Genital pore on anterior portion, ventral surface of posterior portion not covered with papillae ... ... *Gastrodiscoides*
   Genital pore on posterior portion, ventral surface of posterior portion covered with papillae ... ... *Gastrodiscus*

Genus *Gastrodiscus*, Leuckart, 1877.

Definition.—Gastrodiscidae : anterior portion small and conical, posterior portion large and flat ; genital pore on posterior portion, ventral surface of posterior portion covered with papillae.

Type species *Gastrodiscus aegyptiacus* (Cobbold, 1877), Looss, 1896.

Key to Species

Genital pore less than 1 mm. from anterior border of posterior portion *G. aegyptiacus*
Genital pore over 1 mm. from anterior border of posterior portion *G. secundus*

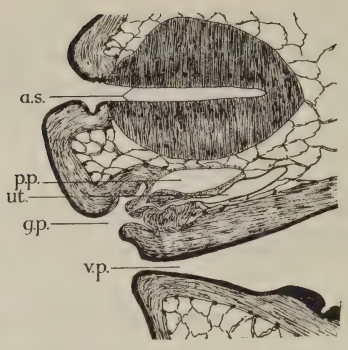

Fig. 22. *Carmyerius exoporus*, n.sp. Sagittal section on enlarged scale to show genital pore. *a.s.*—anterior sucker; *g.p.*—genital pore; *p.p.*—pars prostatica; *ut.*—uterus; *v.p.*—ventral pouch. × 30.

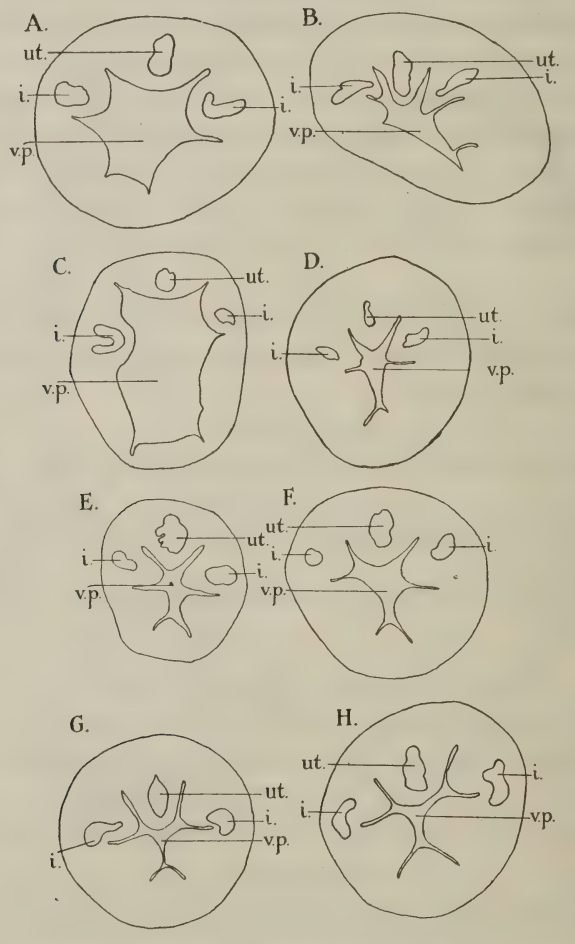

Fig. 23. *Carmyerius exoporus*, n.sp. Transverse section of eight specimens near the middle showing variation in shape of ventral pouch. *i.*—intestine; *ut.*—uterus; *v.p.*—ventral pouch. × 9.

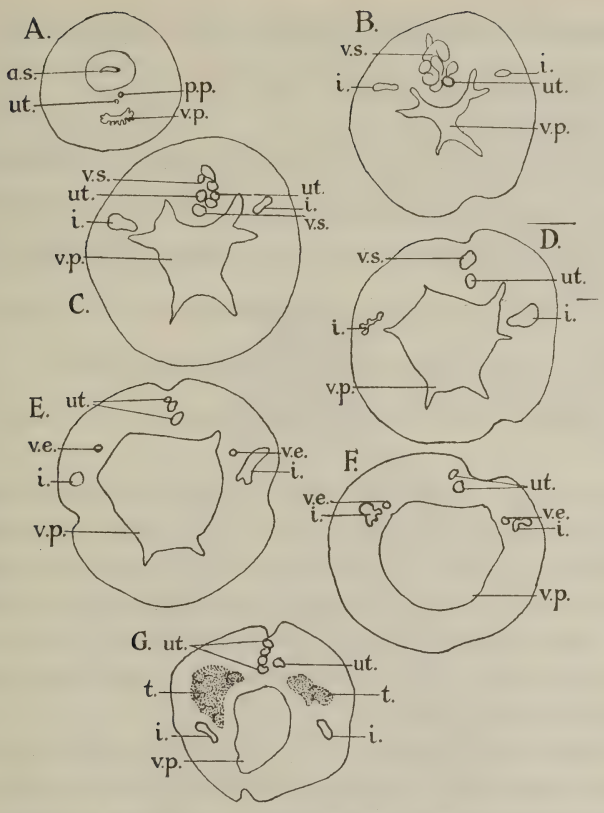

Fig. 24. *Carmyerius exoporus*, n.sp. Seven transverse sections of a single worm at different levels showing alteration in shape of ventral pouch. *a.s.*—anterior sucker; *i.*—intestine; *p.p.*—pars prostatica. *t.*—testes; *ut.*—uterus; *v.e.*—vas efferens; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis. × 12.

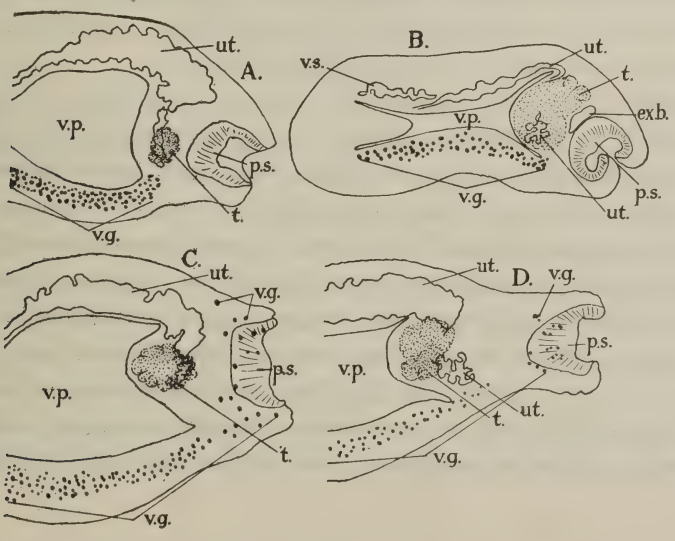

Fig. 25. *Carmyerius exoporus*, n.sp. Sagittal sections of four specimens to show differences in shape of ventral pouch. *A*, *C*, and *D*—Gravid worms. *B*—Immature worm. *ex.b.*—excretory bladder; *p.s.*—posterior sucker; *t.*—testis; *ut.*—uterus; *v.g.*—vitelline gland; *v.p.*—ventral pouch; *v.s.*—vesicula seminalis. × 9.

Gastrodiscus aegyptiacus (Cobbold, 1877), Looss, 1896.

Synonymy :—*Gastrodiscus minor*, Leiper, 1913.

First found in the horse.

The material available consisted of the following collections :—

1. Over one hundred specimens from the large intestine of a pony at Ilorin, Northern Nigeria.
2. Over one hundred specimens from the large intestine of a zebra (*Equus* sp.) in Rhodesia.
3. Over one hundred specimens in poor condition passed by a horse in Northern Nigeria.
4. About fifty specimens from a horse at Nairobi, Kenya Colony.
5. Six specimens from a mule at Nairobi.
6. Three collections from wart-hogs (*Phacochoerus* sp.) in Ngoa, North-East Rhodesia.

As the first four collections consisted mainly of relatively large worms (about 15 mm. in length), and the last four mainly of smaller worms (about 9-12 mm.), it was at first thought that two distinct species were present, but it was found on detailed examination that no differences in anatomy could be discovered, and, as in both types gravid worms were found, it was assumed that the difference is nothing more than a size variation of the one species.

Looss (1896) in his description of *G. aegyptiacus* states that the testes are arranged diagonally, with the anterior of the two on the right side and the posterior on the left ; the ovary was on the left side behind the posterior testis. Among specimens examined in the present instance, it was found that the relative position of the testes varied and that sometimes the left testis was anterior. It was also noted that the ovary was invariably on the same side as the anterior testis.

Vitellaria. The vitelline glands are described by Looss as composed of two groups of follicles arranged near the ventral surface, and along the outer side of the gut caeca in the posterior disc-like portion of the worm, and confined to these two areas, except that in some cases they may spread inwards so as nearly to meet on the dorsal side of the hinder ends of the gut caeca (this arrangement is shown by the heavy dots in fig. 26). Among the present collections of worms, the restricted type of distribution of the vitellaria was noted in two collections only, viz., that from the pony, Ilorin, and from the zebra, Rhodesia. In all the other collections the vitellaria were found to be much more extensive, though somewhat variable in distribution. In fullest development the vitellaria not only extended right

across the dorsal surface of the posterior disc-like part for its whole length, but also into the cephalic portion, where at times they were very thickly massed and completely encircled this part of the worm (fig. 26). The number of gland groups varied very much in different individuals, especially in the cephalic part of the worm. In a few cases the vitellaria in the caudal part of the worm were almost completely confined to the inter-caecal field on the dorsal aspect. In these worms the vitellaria extended

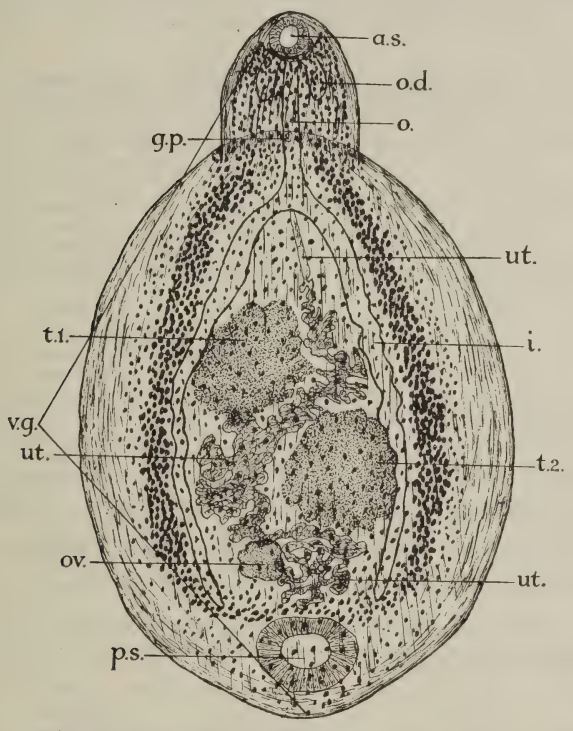

Fig. 26. *Gastrodiscus aegyptiacus.* Dorsal surface uppermost. *a.s.*—anterior sucker; *o.*—oesophagus; *o.d.*—oral diverticulum; *g.p.*—genital pore; *i.*—intestine; *ov.*—ovary; *p.s.*—posterior sucker; *t1.*—anterior testis; *t2.*—posterior testis; *ut.*—uterus; *v.g.*—vitelline gland. × 6.

as a single broad band from the anterior end to the posterior end of the worm. It might be considered that these differences are of specific value, but in view of the great variation found in the distribution of the vitellaria in all other species of *Amphistomata* that the writer has examined, one is compelled to consider them of no specific value.

With the object of determining the exact position of the genital pore in relation to the anterior edge of the caudal portion of the worm, nineteen

specimens from the various collections were examined (see Table X). It was not possible to examine more specimens in this particular point, because the edge of the caudal part is usually incurved, so that it requires considerable pressure, and hence distortion, to flatten it out sufficiently to measure the distance between the genital pore and this edge.

Table X

Distance between genital pore and anterior edge of discal portion of worm

Host	Type of worm (large or small)	Distance of genital pore from anterior edge of caudal part	Total length of discal part	Ratio between the two foregoing dimensions
Wart-Hog	Small	572μ	9·9 mm.	1 : 17·3
		625μ	11·0 mm.	1 : 17·6
		416μ	9·1 mm.	1 : 21·8
		416μ	8·9 mm.	1 : 21·4
Wart-Hog	Small	468μ	10·0 mm.	1 : 21·4
		781μ	11·4 mm.	1 : 14·6
		572μ	10·4 mm.	1 : 18·2
		625μ	9·8 mm.	1 : 15·6
		400μ	8·3 mm.	1 : 20·75
Mule, Nairobi	Small	468μ	8·8 mm.	1 : 18·9
		572μ	9·6 mm.	1 : 16·8
Zebra	Large	781μ	13·3 mm.	1 : 17·1
		729μ	13·0 mm.	1 : 17·8
Pony, Ilorin	Large	760μ	13·5 mm.	1 : 17·8
Horse, Nairobi	Large	625μ	11·3 mm.	1 : 18·1
		833μ	12·5 mm.	1 : 15·0
		677μ	12·0 mm.	1 : 17·7
		639μ	11·25 mm.	1 : 17·8
		625μ	13·75 mm.	1 : 22·0

It will be noted from the above table that the distance between the genital pore and the anterior edge of the caudal part of the worm varies considerably. Another point which this series of measurements brought out is that although on the whole the two types are fairly distinct, relatively small worms may be found in bottles in which the predominating number are large, and in bottles that contain nearly all small worms a few relatively large ones occur, and these exceptional specimens tend to unite the two types into a complete whole. From these observations it is

concluded that the species *G. aegyptiacus* is subject to considerable size variation, and that the distance of the genital pore, although further from the edge of the caudal part of the worm in large examples than it is in small ones, is variable.

Gastrodiscus minor, Leiper, 1913.

This species is recorded by Leiper (1913) as new, the host being the pig in Uganda. All Leiper gives in the way of description is the following passage :

'This small fluke resembles closely the African *G. aegyptiacus* (*vel sonsinoi*), which is so frequently met with in horses in Egypt and in West Africa : it differs, however, in a number of respects, particularly in the nearness of the genital pore to the edge of the ventral disc-like expansion.'

From this it is rather difficult to deduce in what the differences between *G. minor* and *G. aegyptiacus* consist, especially if reference is made to Table X. *G. minor* is therefore regarded as a synonym of *G. aegyptiacus*.

Gastrodiscus secundus, Looss, 1907.

The material available for study consisted of a single collection of about twenty specimens. It was from the same collection that Looss obtained the material he used in his description of the species.

Examination of this material did not reveal any differences from the original description by Looss (1907), except that the position of the testes and ovary varied in the same manner as has been described in *G. aegyptiacus*. The worm can at once be distinguished from *G. aegyptiacus* by the position of the genital pore. Fig. 27 shows the essential anatomical details.

Genus *Gastrodiscoides*, Leiper, 1913.

Definition.—Gastrodiscidae : anterior portion small and conical, genital pore on anterior portion, no papillae on ventral surface of posterior portion.

Type species *Gastrodiscoides hominis* (Lewis and McConnal, 1876), Leiper, 1913.

Only one species described.

Synonymy :—

Amphistoma hominis, Lewis and McConnal, 1876.
Gastrodiscus hominis, Ward, 1903.

First found in the colon of man in Assam.

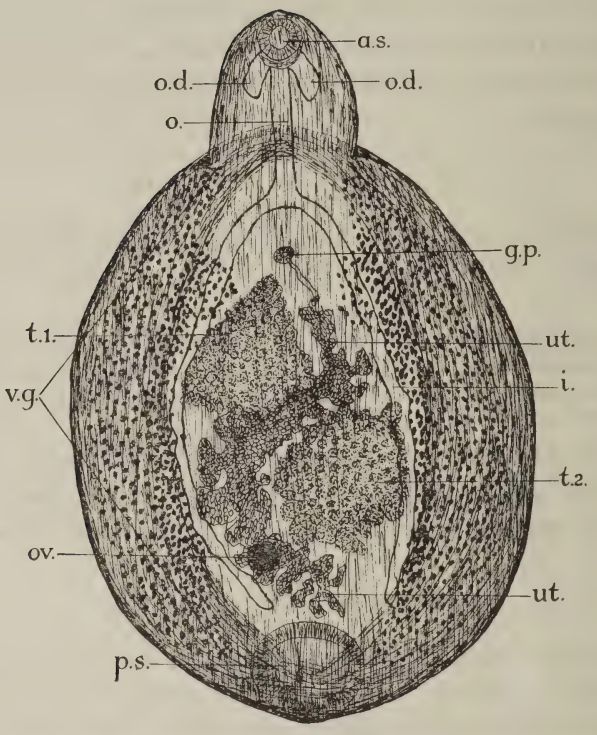

Fig. 27. *Gastrodiscus secundus.* Dorsal surface uppermost. *a.s.*—anterior sucker; *g.p.*—genital pore; *i.*—intestine; *o.*—oesophagus; *o.d.*—oral diverticulum; *ov.*—ovary; *p.s.*—posterior sucker; *t1.*—anterior testis; *t2.*—posterior testis; *ut.*—uterus; *v.g.*—vitelline gland. × 12.

Gastrodiscoides hominis (Lewis and McConnal, 1876), Leiper, 1913.

The material available for study consisted of :—

Two collections from Annam consisting of nine whole worms and six specimens cut and mounted in serial section. This is the same material as Stephens (1906) used in his description of the worm.

Leiper describes and figures (fig. 35) a prominent genital papilla with the male and female ducts opening separately near its tip. Figs. 28 and 29 in the present paper were drawn from two sectioned specimens cut by Stephens. In fig. 28, it will be noted that there is a deep atrium with no sign of a papilla, the openings of the uterus and vas deferens being widely separated from one another and lying at the deepest part of the atrium. This represents a worm with the papilla completely retracted. In fig. 29 there is a bulbous papilla partly protruded, with a small atrium surrounding

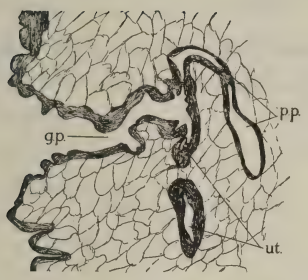

Fig. 28. *Gastrodiscoides hominis.* Sagittal section of genital pore, with papilla fully retracted. *g.p.*—genital pore; *p.p.*—pars prostatica; *ut.*—uterus × 30.

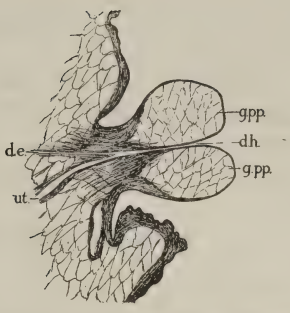

Fig. 29. *Gastrodiscoides hominis.* Sagittal section of genital pore, with papilla partly extruded. *d.e.*—ductus ejaculatorius; *d.h.*—ductus hermaphroditicus; *g.pp.*—genital papilla; *ut.*—uterus. × 30.

its base, the male and female ducts uniting within the papilla and opening at its tip by a common duct, in the usual way. These two drawings taken in conjunction with Leiper's figure indicate that the presence or absence of a prominent genital papilla, or of a genital atrium, are purely matters of chance, and are of no more diagnostic value in this instance than in any other species of the group *Amphistomata.*

Genus *Homalogaster,* Poirier, 1883.

Definition.—Gastrodiscidae: anterior portion large and flat, posterior portion smaller and spherical.

Type species *Homalogaster poloniae,* Poirier, 1883.

Only one species described.

Homalogaster poloniae, Poirier, 1883.

Synonymy :—

Homalogaster poirieri, Giard and Billet, 1892.

Homalogaster philippinensis, Stiles and Goldberger, 1910.

First found in stomach of *Palonia frontalis,* in Java.

The writer has not had the opportunity of examining any material of this species, but Railliet, Henry, and Bauche (1914) discuss its synonymy, and it is from their paper that it has been taken.

Brumptia, Travassos, 1921

Definition.—Amphistomata : with paired caudal appendages containing most of the vitellaria, cirrus pouch and genital sucker present.

Type species *Brumptia gigas.*

Brumptia gigas (MacCallum, 1917) Travassos, 1921

Synonymy :—

Cladorchis gigas, MacCallum, 1917.

This worm was found on two occasions in the stomach of a rhinoceros at Ngoa, North-east Rhodesia.* Each collection consists of about twenty-five specimens.

External Anatomy

Size and shape. The worms were of slightly different size in the two collections, those in one bottle being all about 15 mm. in length by 9 mm. in breadth, and those in the other bottle about 12 mm. in length by 7 mm. in breadth. Gravid worms were found in both collections, but as detailed examination revealed no differences other than size, it is considered that there was only one species. The worms consist of two portions distinctly separated, an anterior conical portion and a posterior part consisting of two crescentic flaps. The anterior part is conical in shape with a definite ventral curve ; the ventral surface is almost flat from side to side, whilst the dorsal surface is domed both laterally and antero-posteriorly.

The posterior sucker is slightly in front of the posterior extremity of the body of the worm, and is situated entirely on the ventral surface, and directed ventrally. About midway between the two suckers in the mid-line of the ventral surface is the genital pore. Comparison of figs. 5 and 6, Plate VIII, will show how the appearance of the genital pore varies with retraction or extrusion of the genital papilla.

The most characteristic feature of the worm is the presence of two large

* The above description was written before I became aware that MacCallum (1917) had described a worm which is apparently the same species. MacCallum obtained his material from the African elephant (*Loxodon africanus*), he named it *Cladorchis gigas*. The Liverpool material seems to be identical with MacCallum's in all anatomical details, but the two collections are a little different in size, MacCallum's worms are 21 mm. in length, and ours are from 12 mm. to 15 mm. in length. In view of the results obtained in other species this slight difference is not considered to be of importance. Travassos (1921) created a new genus *Brumptia* to accommodate MacCallum's species *C. gigas*, leaving it in the sub-family *Cladorchinae*, but the two caudal flaps containing the vitelline glands are so strikingly different from any other genus in this sub-family that I consider it preferable to leave it as a genus of uncertain position.

crescentic caudal appendages. These arise from the postero-lateral borders, extend laterally as far forward as the anterior border of the ventral sucker, and are separated from one another behind the sucker by a deep notch. In full extension they measure about 5 mm. in length, but as a rule, their borders are incurved towards the ventral surface, so that they appear somewhat shorter and tend to overlap the posterior sucker.

Internal Anatomy

On account of the thickness of these worms, very little could be ascertained in whole specimens cleared in carbolic acid, so that the following description is mainly based on a study of serial sections cut in sagittal, coronal, and transverse planes.

Muscular system. The muscular system, as a whole, is very similar to other species, but in certain special structures it departs from the usual type, and these differences will be dealt with under the appropriate organs.

Nervous system. This system was not investigated.

Excretory system. The excretory bladder is large when in a state of distension and occupies the whole of the posterior part of the worm between the posterior sucker and the dorsal surface. The excretory canal in the specimens examined ran almost directly posteriorly to open in the mid-line near the posterior end of the dorsal surface (fig. 30, B).

Anterior sucker. The anterior sucker is a thick walled muscular structure surrounding the oral cavity ; about the junction of the middle and posterior thirds there is an annular constriction at which point two large muscular diverticula arise and run in a dorsal and slightly posterior direction (figs, 31, A and 32, C).

Oesophagus and intestines. The oesophagus is of the usual type, its muscular wall becoming slightly thicker as the posterior end is approached. In the specimens examined, it curved at first ventrally, and then turning abruptly towards the dorsal surface, divided into the gut caeca in front of the cirrus pouch (fig. 30, B). The caeca pursue a wavy course along each side of the worm and end in the dorsal part of the caudal flap (fig. 30, A).

Genitalia. Testes. These are large oval organs lying side by side in the lateral fields somewhat nearer to the ventral than to the dorsal surface. They lie in front of the posterior sucker, and the posterior part of the cirrus pouch is between their anterior ends (figs. 30, A, 31, B, and 32, B). No external lobing is visible, but in sections each testis is seen to be divided

up into numerous separate acini, the whole being surrounded by a loose connective tissue capsule. Each testis is about 3·5 mm. in diameter.

Vasa efferentia. These arise from the antero-mesial aspect of each testis, and suddenly dilate into broad thin-walled tubes, which, running upwards and inwards over the posterior wall of the cirrus pouch, enter this structure on its dorso-posterior aspect by two narrow tubes lying close to each other. When they reach the inner aspect of the wall of the cirrus pouch they unite to form the vas deferens (figs. 30, 31, C and 32, A).

Vas deferens. The vesicula seminalis is a dilated, thin-walled sac

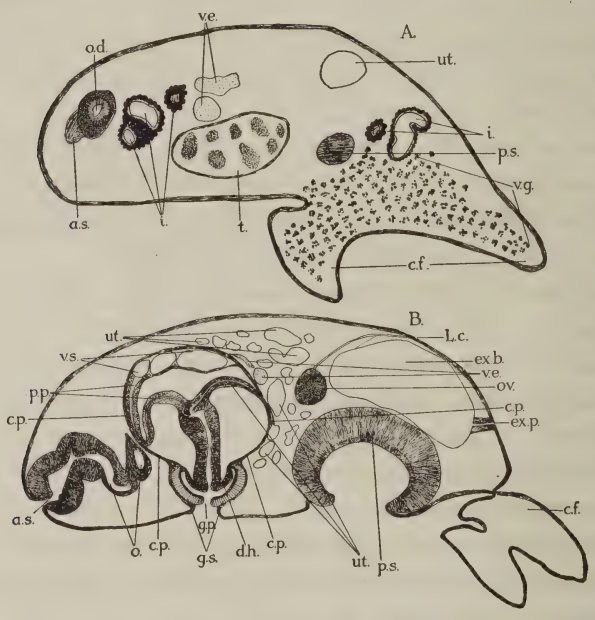

Fig. 30. *Brumptia gigas.* Sagittal sections. *A*—To one side of mid-line. *B*—In mid-line. *a.s.*—anterior sucker; *c.f.*—caudal flap; *c.p.*—cirrus pouch; *d.h.*—ductus hermaphroditicus; *ex.b.*—excretory bladder; *ex.p.*—excretory pore; *g.p.*—genital pore; *g.s.*—genital sucker; *L.c.*—Laurer's canal; *o.*—oesophagus; *o.d.*—oral diverticulum; *ov.*—ovary; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *t.*—testis; *ut.*—uterus; *v.e.*—vas efferens; *v.s.*—vesicula seminalis. × 6.

which runs along the dorsal wall of the cirrus pouch, being held in place by some strands of connective tissue (figs. 30, B and 31, B). Near the anterior end of the cirrus pouch on its dorsal surface, the vesicula seminalis passes into the pars prostatica. The pars prostatica runs ventrally for some distance close along the anterior wall of the cirrus pouch, and then leaving the wall of the cirrus pouch turns sharply towards the dorsal surface, curving posteriorly until it ends by uniting with the uterus near the centre of the pouch (fig. 30, B). The pars prostatica is thickly

surrounded by cells for its whole course and no pars musculosa could be distinguished. The genital papilla appeared as a long muscular tube running from about the centre of the cirrus pouch towards the ventral surface (figs. 30, B and 31, B), but in all the specimens sectioned it was in a state of retraction, and would probably appear quite different in sections of a worm like that shown in Plate VIII, fig. 5, where it is obviously protruded.

Cirrus pouch. The cirrus pouch is a spherical organ about 4 mm. in diameter. It lies near the centre of the worm slightly towards its anterior end. Its wall is composed of loosely laminated muscular fibres. That part of the cirrus pouch which is not occupied by sex ducts is filled with loose areolar tissue.

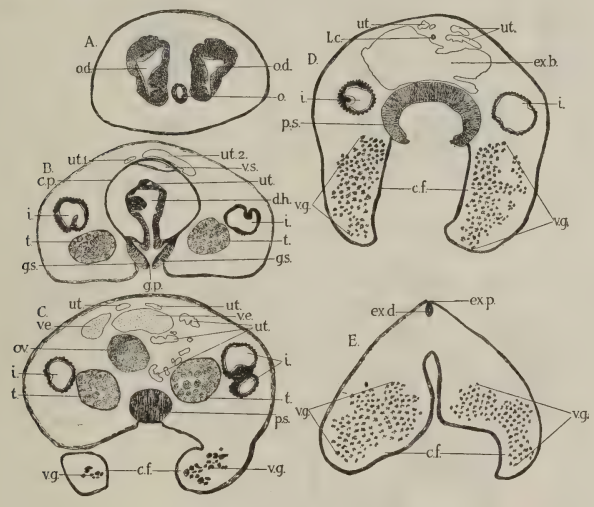

FIG. 31. *Brumptia gigas.* Transverse sections. *A*—Near anterior end. *B*—Through cirrus pouch. *C*—Through ovary. *D*—Through middle of posterior sucker. *E*—Through excretory pore. *c.f.*—caudal flap; *c.p.*—cirrus pouch; *d.h.*—ductus hermaphroditicus; *ex.b.*—excretory bladder; *ex.d.*—excretory duct; *ex.p.*—excretory pore; *g.p.*—genital pore; *g.s.*—genital sucker; *i.*—intestine; *L.c.*—Laurer's canal; *o.*—oesophagus; *o.d.*—oral diverticulum; *p.s.*—posterior sucker; *t.*—testis; *ut.*—uterus; *ut1.*—ascending branch of uterus; *ut2.*—descending branch of uterus; *v.e.*—vas efferens; *v.g.*—vitelline gland; *v.s.*—vesicula seminalis. × 4¼.

Genital pore. This is provided with a definite small sucker surrounding its opening. This sucker is much more definitely marked off from the subcuticular muscle than in the case of the genus *Cotylophoron*, as it is composed of radially arranged fibres quite distinct from the subcuticular muscle; this is shown in figs. 31, B and 30, B, in both of which the genital papilla is seen lying within the genital sucker and surrounded by a small atrium, which would obviously disappear if the papilla were extruded.

Ovary. This lies towards the dorsal surface between the testes and slightly to one side of the mid-line. It is a circular organ with no special characters (figs. 30, B, 31, C, and 32, A). The shell gland lies on the mesial aspect of the ovary (fig. 32, A).

Laurer's canal. Laurer's canal runs dorsally from the shell gland and, curving posteriorly over the anterior end of the excretory bladder, it opens in the mid-line above the middle of the bladder and far in front of the excretory pore (fig. 30, B).

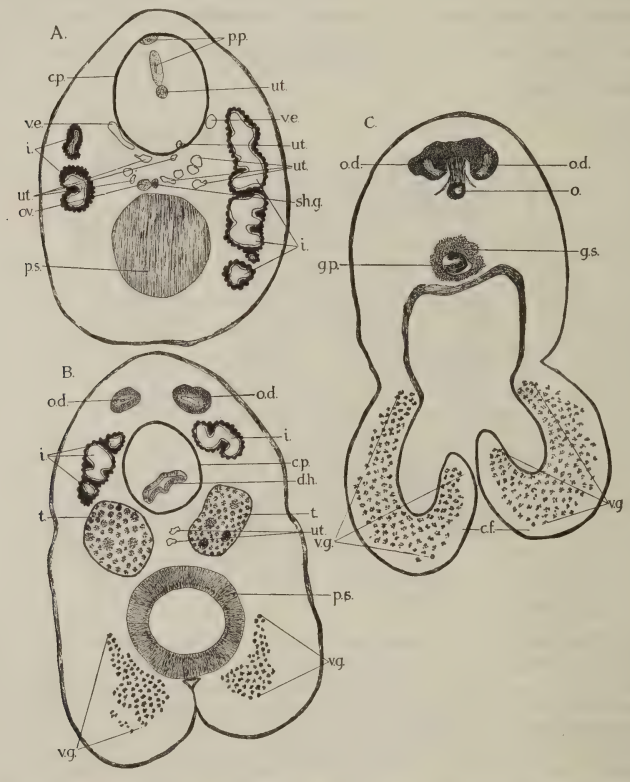

FIG. 32. *Brumptia gigas.* Coronal sections. *A*—Towards dorsal surface. *B*—About the middle. *C*—Near the ventral surface. *c.p.*—cirrus pouch; *d.h.*—ductus hermaphroditicus; *g.p.*—genital pore; *g.s.*—genital sucker; *i.*—intestine; *o.*—oesophagus; *o.d.*—oral diverticulum; *ov.*—ovary; *p.p.*—pars prostatica; *p.s.*—posterior sucker; *sh.g.*—shell gland; *t.*—testis; *ut.*—uterus; *v.e.*—vas efferens; *v.g.*—vitelline gland. × 4½.

Vitellaria. The vitelline glands consist of numerous collections of follicles which lie nearly entirely within the two caudal appendages (figs. 30, A, 31, C. D and E, 32, B and C). A few groups of follicles were seen near the ventral surface of the worm in front of the posterior sucker.

Uterus. For the first part of its course the uterus shows no special differences from the usual type. But after it reaches the anterior border of the cirrus pouch on its dorsal aspect, it turns posteriorly and runs back, still close to the dorsal wall of the cirrus pouch, and following the curve of the posterior wall of this organ comes close to the ventral surface ; it then turns sharply dorsally and enters the posterior wall of the cirrus pouch about its middle. From this point, it runs anteriorly through the pouch to unite near the centre with the end of the pars prostatica (fig. 30, B).

Eggs. The eggs removed from the uterus of one specimen were oval and operculated and measured 112μ to 116μ in length by 76μ to 70μ in breadth, but it must be remembered that measurements of eggs taken from the uterus of fixed worms are only very approximate.

SPECIES INQUIRENDAE

Amphistomum papillatum, Cobbold, 1882.

Found in intestine of *Elephas indicus,* India.

Amphistomum tuberculatum, Cobbold, 1875.

Found in intestine of *Bos taurus,* India.

Amphistomum emarginatum, Diesing, 1839.

Found in intestine of *Nictipithecus trivirgatus,* Brazil.

CONCLUSION

As a result of an exhaustive examination of a very large collection of material comprising in many instances some hundreds of specimens, and of a careful and critical study of the monographs of Fischoeder and of Stiles and Goldberger, the conclusion is reached that many of the species described by the former, and all except one of those described by the latter authors, are merely synonyms of earlier species. It appears to the writer that the authors have fallen in error owing to the fact that they confined themselves to the examination of limited material, in some cases to the examination of a single non-gravid worm, or even in one or two instances to that of a series of sections of a single specimen. It is only when a long series of specimens is examined that one realises to what extent individual variations occur.

LIST OF AMPHISTOMES ARRANGED UNDER THEIR HOSTS

Host	Parasite	Location
Manatus exunguis	*Chiorchis fabaceus*	Intestine.
„ *latirostris*	„ „	„
Tapirus americanus	*Cladorchis asper*	„
	Cladorchis pyriformis	„
Equus caballus	*Gastrodiscus aegyptiacus*	„
	Pseudodiscus collinsi	„
	Gastrodiscus secundus	„
„ *zebra*	„ *aegyptiacus*	„
„ *mulus*	„ „	„
Rhinoceros sp. (Rhodesia)	... *Brumptia gigas*	?
Phacochoerus sp. ... ... (North-East Rhodesia)	... *Gastrodiscus aegyptiacus*	„
Sus sp. (Annam.) ...	... *Gastrodiscoides hominis*	„
Dicotyles albirostris ...	... *Cladorchis giganteus*	„
„ *labiatus*	„ „	„
„ *torquatus*	„ „	„
	Taxorchis schistocotyle	„
Hippopotamus amphibius	... *Paramphistomum gigantocotyle* ...	Stomach.
	„ *wagandi* ...	„
	„ *buxifrons* ...	„
	Cotylophoron cotylophorum ? ...	„
	„ *minutum*	„
	Carmyerius cruciformis	„
	Paramphistomum pisum	Intestine.
Bos taurus	*Paramphistomum cervi*	Stomach.
	„ *explanatum* ...	„
	„ *orthocoelium* ...	„
	Cotylophoron cotylophorum ...	„
	Stephanopharynx compactus ...	„
	Gastrothylax crumenifer	„
	Fischoederius cobboldi	„
	„ *elongatus*	„
	Carmyerius gregarius	„
	„ *spatiosus*	„
	Homalogaster poloniae	Large intestine.
	Amphistomum tuberculatum ? ...	Intestine.
Bos taurus indicus	*Paramphistomum cervi*	Stomach.
	„ *orthocoelium* ...	„
	Cotylophoron cotylophorum ...	„
	Gastrothylax crumenifer	„
	Fischoederius cobboldi	„
	„ *elongatus*	„
	Carmyerius spatiosus	„
	Paramphistomum explanatum ...	Bile ducts.
Bos urus	„ *cervi*	Stomach.
Bos. sp. (Pagan dwarf bull), Ilorin	*Cotylophoron cotylophorum* ...	„
Bos bubalus (*Bison europaeus*)	*Paramphistomum cervi*	„
	Carmyerius gregarius	„

Bos (*bubalus*) *caffer*, Africa	*Cotylophoron cotylophorum*	Stomach.
	Carmyerius gregarius	,,
Bos (*bubalus*) *bubalis*, Asia	*Paramphistomum cervi*	,,
	Carmyerius gregarius	,,
Palonia frontalis	*Fischoederius cobboldi*	,,
	,, *elongatus*	,,
	Homalogaster paloniae	Caecum.
Anoa depressicornis	*Fischoederius elongatus*	Stomach.
Capra hircus	*Paramphistomum cervi*	,,
,, sp. (India)	*Gastrothylax* sp. ? (immature)	,,
,, sp. (Northern Territory, Gold Coast)	*Paramphistomum* sp. ? (immature)	,,
Ovis aries	*Paramphistomum cervi*	,,
,, sp. (Port Said)	,, ,,	
	Cotylophoron cotylophorum	,,
	Gastrothylax sp. ? (immature)	,,
,, sp. (South Africa)	*Paramphistomum* sp. ? (immature)	,,
,, sp. (Hong Kong)	*Paramphistomum orthocoelium*	,,
	Gastrothylax crumenifer	,,
Antilope dorcas	*Paramphistomum cervi*	,,
Antilope sp. (Kamerun)	*Carmyerius spatiosus*	,,
Cobus sp. (North-east Rhodesia)	*Stephanopharynx compactus*	,,
	Cotylophoron cotylophorum	,,
Cobus sp. (Zeref)	*Cotylophoron cotylophorum*	,,
Cobus maria	*Carmyerius wenyoni*	,,
Tragelaphus scriptus	*Carmyerius spatiosus*	Stomach ?
,, *spekei*	,, *exoporus*	Stomach.
Hippotragus equinus	*Paramphistomum cervi*	,,
,, ,,	*Carmyerius spatiosus*	,,
Aepyceros melampus	*Cotylophoron cotylophorum*	,,
Bubalus sp. (Nyasaland)	,, ,,	,,
	Paramphistomum explanatum	
Bubalis sp. (Rhodesia)	*Carmyerius spatiosus*	,,
Cervicapra sp. (Rhodesia)	,, ,,	,,
Portax tragocamelus	*Paramphistomum cervi*	,,
Cervus alces	,, *cervi*	,,
,, *campestris*	,, *liorchis*	,,
,, *capreolus*	,, *cervi*	,,
,, *dama*	,, ,,	,,
,, *dichotomus*	,, *liorchis*	,,
	Balanorchis anastrophus	,,
	Amphistomum lunatum ?	Intestine.
,, *elaphus*	*Paramphistomum cervi*	Stomach.
,, *mexicanus*	,, *liorchis*	,,
,, *namby*	,, ,,	,,
,, *rufus*	,, ,,	,,
,, *simplicicornis*	,, ,,	,,
Elephas indicus	*Pseudodiscus hawkesii*	Intestine.
Loxodon africanus	*Brumptia gigas*	,,
	Amphistomum papillatum ?	,,
Castor fiber	*Cladorchis subtriquetrus*	Small and large Intestine.

Callithrix noctivaga ...	... *Amphistomum emarginatum* ?	... Intestine.
Cercopithecus callitrichus	... *Pseudodiscus watsoni* ...	... Colon.
Macacus cynomolgus ...	... *Pseudodiscus watsoni* ? ...	... Colon.
Homo sapiens	... *Gastrodiscoides hominis* ...	... Intestine.
	Pseudodiscus watsoni	

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EXPLANATION OF PLATE V

Paramphistomum cervi. Photographs showing variations in size and shape exhibited by 24 specimens. × $2\frac{1}{2}$.

FIG. A

FIG. B

EXPLANATION OF PLATE VI

Fig. A. *Paramphistomum explanatum.* × 2½.

Fig. B. *Cotylophoron cotylophorum.* × 2½.

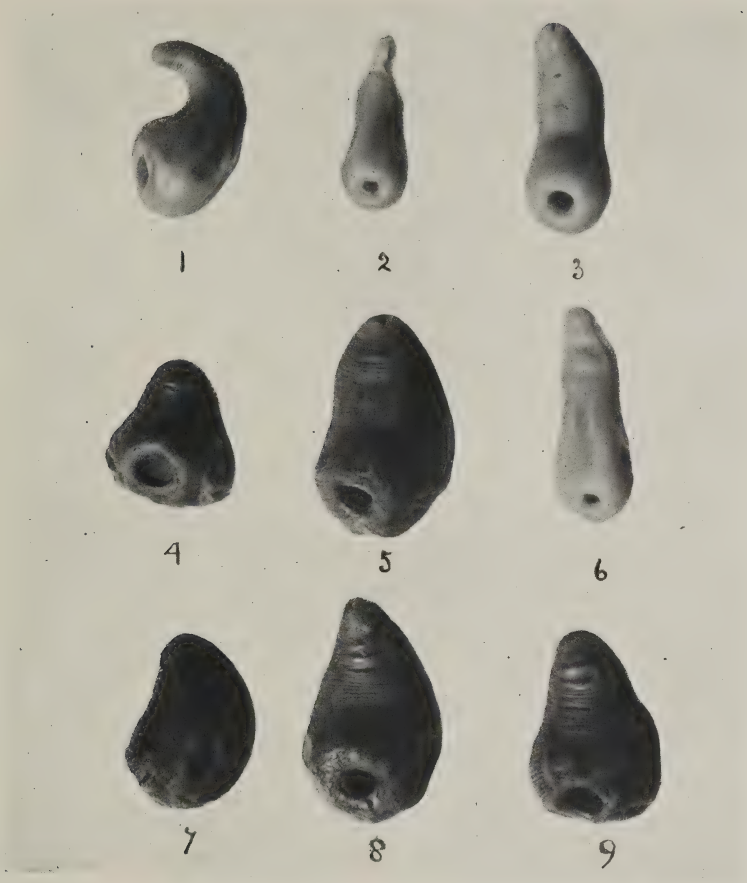

FIG. A

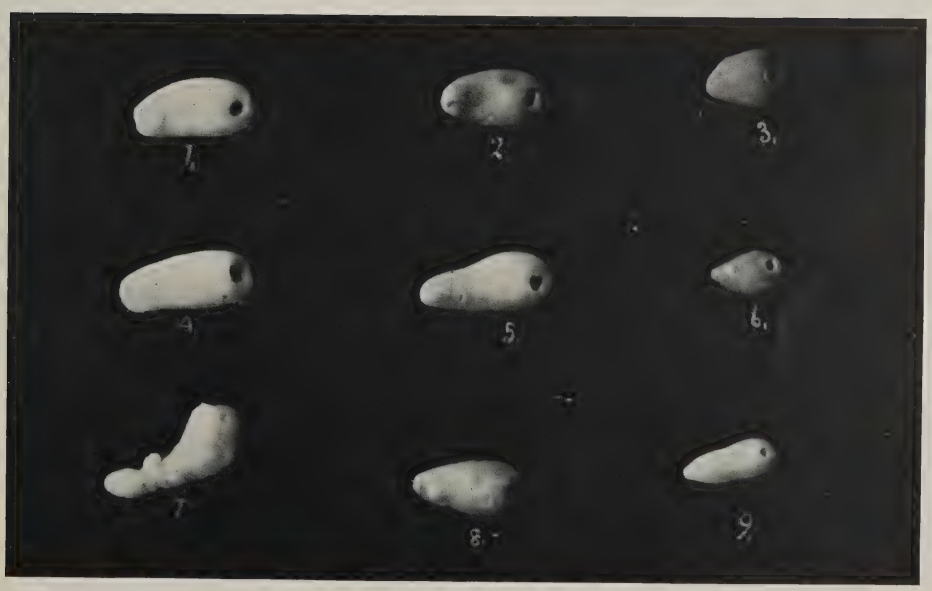

FIG. B

EXPLANATION OF PLATE VII

Fig. A. *Carmyerius exoporus*, n.sp. × 2½.

Fig. B. *Stephanopharynx compactus*. × 2½.

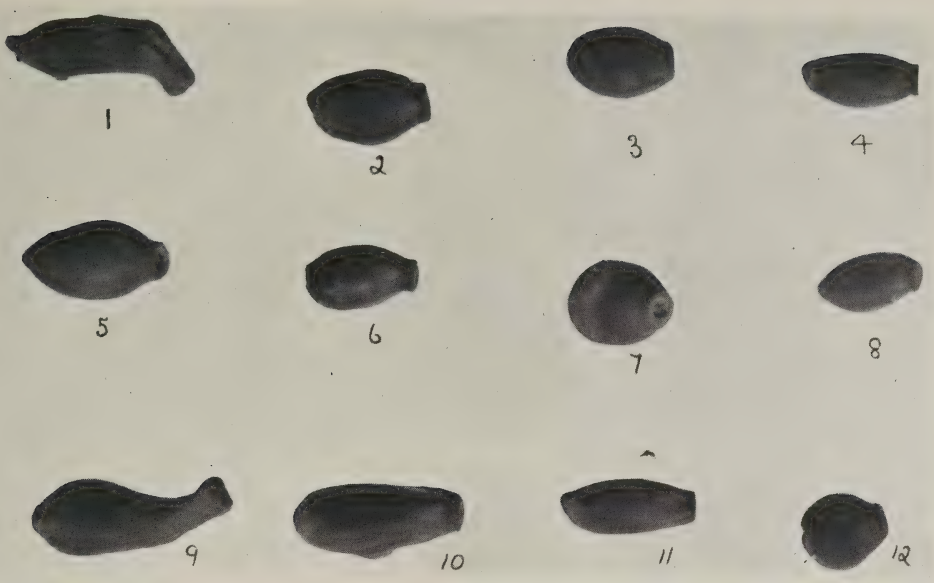

FIG. A

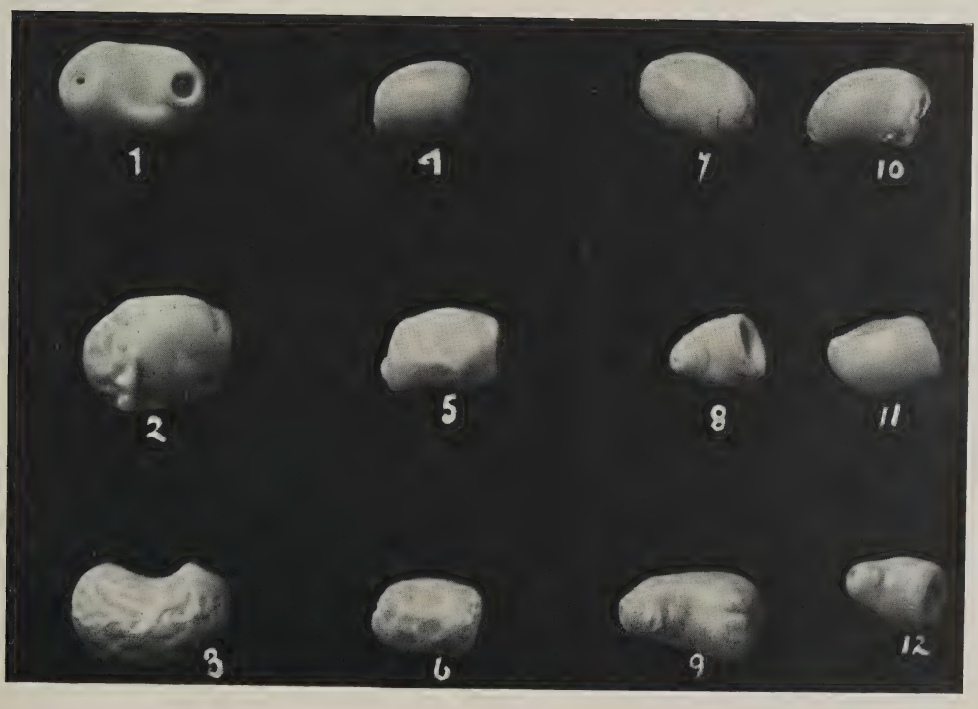

FIG. B

EXPLANATION OF PLATE VIII

Brumptia gigas

Figs. 1 and 2. Dorsal view.

Figs. 3 and 4. Lateral view.

Figs. 5 and 6. Ventral view. (5) Genital papilla extruded; (6) genital papilla retracted. × $2\frac{1}{2}$.

PLATE VIII

MALARIA IN AUSTRALIA

BY

P. A. MAPLESTONE

(*Received for publication* 25 *February*, 1923)

The following is a short account of the history of malaria in Australia as far as it can be collected from the published records. In compiling this review all the information prior to the year 1912 has been taken from Cleland (1914); since 1912 the original articles in the medical press and the various Government reports have been consulted. This is not a complete discussion of all the references to malaria in Australia; many of the earlier accounts are given by laymen, or are merely the expression of an opinion by a medical man without definite proof, and a considerable number of the later records are purely of local interest, so they have been ignored. In discussing the subject, the various States and the Northern Territory of the Commonwealth are considered separately.

QUEENSLAND

According to Cleland (1914), the first account of malaria in Australia by a medical man is that of White (1867). In all probability this is the same outbreak as the one to which Elkington (1912) refers, and which he concludes was introduced to Burketown by a ship from Java. Cleland mentions that for some years prior to 1885 there was extensive and severe malaria in North Queensland. In support of this statement he quotes the following authorities, viz.:—A'Hearne (1890) for Townsville, Graham-Browne (1890) for Charters Towers, Hunt (1890) for Hughenden, and James (1891) for Croydon. But from the quotations taken by

Cleland from these authors' writings it is by no means clear that all the epidemics included in this series were due, altogether or even partly, to malaria. For instance, there is no way of finding out if all A'Hearne's cases were malaria ; Graham-Browne's description of the Charters Towers epidemic is unlike malaria ; Hunt obviously confuses typhoid fever and malaria, and James under the term ' Gulf fever ' describes all his febrile cases, some of which were probably malaria. For the same period Jeffris-Turner says he only saw three cases of malaria in children in Brisbane. Whatever these various outbreaks were, it is a striking fact that since the advent of more accurate diagnostic methods, malaria has not been recorded from any of the above towns, but enteric fever is fairly often encountered there.

From Cleland's account it appears that O'Brien (1908) was the first to record finding the malaria parasites in Australia ; nearly all his cases were simple tertian, but he writes of finding a few quartan and malignant tertian. O'Brien's observations were apparently made at Yarabah mission station near Cairns. Breinl (1911), without quoting an authority and after only a few months in the country, reported malaria to be epidemic in ' parts of Queensland,' mentioning specifically Innisfail, Cooktown, and Saxby River. Nevertheless, he evidently saw some malaria, as he adds that the locally acquired cases were simple tertian, and that the few cases of malignant tertian that he saw were infected in Papua (=British New Guinea).

Elkington (1912) refers to a localised epidemic of malaria, which occurred at Kidston on the Einasleigh gold field in 1910. There were 120 cases and 24 deaths in a population of 400. The outbreak was investigated by Dr. Baxter-Tyrie, who concluded that the disease had been introduced from New Guinea. From the latter's report Elkington concludes that malignant malaria and blackwater fever are endemic ; there is no further reference to this ' endemic ' centre in the medical literature nor in the Government reports, therefore it is clear that Elkington's conclusion was premature. Neither is there any evidence that malaria spread from Kidston to any of the surrounding camps.

Although a few cases of malaria undoubtedly occur annually in the coastal districts of North Queensland, there is no way of finding out their numbers. In the Annual Health Report for the State, acute malaria first appears as a notifiable disease in the period 1st July, 1915, to 30th June, 1916. The figures up to the present time are given in Table I.

Table I.

Cases of malaria notified in Queensland.

Year	No. of cases
1915–1916	79
1916–1917	213
1917–1918	72
1918–1919	10
1919–1920	9
1920–1921	9
1921–1922	19

Unfortunately there is no way of ascertaining how many of the above cases were contracted in Queensland, and how many came from elsewhere, except that in 1916-1917 the Sanitary Inspector of the Northern District states in his report that 119 cases of malaria occurred in Cairns for that year. It can also be found indirectly, by comparing the above figures with those of the Australian Institute of Tropical Medicine for corresponding periods, that nearly all the remaining cases were returned soldiers who had become infected outside Australia.

Although this information is very incomplete it is quite obvious that the Cairns epidemic was short-lived and that not many cases can be occurring there at the present time. A possible explanation of this short epidemic in Cairns is that this is the first port of call for boats coming from New Guinea to Australia. In the period immediately preceding and during the sudden increase in malaria in this town, large numbers of soldiers returning from New Guinea were calling there, and the majority of them were being sent home because they were suffering from malaria. The extra opportunity for the mosquitoes of Cairns to become infected soon reacted on the local inhabitants ; but in 1918, when traffic between New Guinea and Australia returned to normal and fewer persons with malaria parasites in their blood were calling at Cairns, the incidence of malaria there suddenly dropped and has remained low ever since. It is true that Breinl and Taylor (1918), after a malaria and mosquito survey of the town recommended the filling and draining

of various swamps in and around it ; but the drop in malaria incidence cannot be explained in this manner, because Mr. Hill, Entomologist of the Australian Institute, visited Cairns in 1921 and at the writer's request examined the mosquito-breeding places recorded by Breinl and Taylor in 1918. He reported that little had been done in reducing these breeding places.

Dr. H. H. Willis, in a letter to the writer in May, 1921, informed him that while on a ' hookworm ' survey of the native settlement on the Palm Islands he had found nine or ten* cases of acute malaria which he had diagnosed microscopically. Over a month later the writer visited these Islands ; he examined all the natives (over 300) and found that five or six* of the cases reported by Willis had crescents in their blood. All the other natives were negative on blood examination, no palpable spleens were found although there were numerous children, and no fresh cases had occurred between the visits of Willis and the writer. The evidence of the origin of this small outbreak was not satisfactory, but as far as could be gathered it seemed likely that the malaria had been introduced from the mainland by some recent arrivals. It is remarkable that the outbreak did not spread further, because the natives were living closely congregated in unscreened grass huts, and Hill found Anopheline mosquitoes breeding close to the dwellings (see Table VI).

The history of Townsville during recent years from the point of view of malaria is of considerable interest, because since the establishment of the Australian Institute of Tropical Medicine in 1910, more reliable records are available from there than from any other town in Northern Australia. Many parasite carriers have been constantly arriving in Townsville for treatment during the eleven and a half years January, 1910 to June, 1921, and for the whole of that time no case of malaria has ever been discovered which was contracted in the town or its surroundings. There is also abundant evidence that the same species of Anophelines are found as in other Coastal towns where malaria occurs. Townsville is well within the tropics and is by far the largest town in Northern Australia with a population of about 25,000, nearly all of whom are whites.

* Figures from memory.

THE NORTHERN TERRITORY

Again consulting Cleland (1914) it is found that Wood (1889) said that malaria was very prevalent in the Northern Territory during the years 1879, 1880, and 1881. Holmes (1913) gives the following figures taken from the official records :—

TABLE II.

Deaths in the Northern Territory.

Year	Total Number of deaths	Deaths due to 'fever'
1879	166	61
1880	154	61
1881	100	51

It was at this time that gold-mining was at its height and there were many mining camps in the country with no medical man near them and no sanitary precautions in force. Although many of the deaths were in all probability due to malaria, it should be borne in mind that a large number of the cases could not have been seen by a medical man, so that the diagnosis of 'fever' on the death certificates is not of much use for accurate record.

The Umbrawarra tin mining field was opened up about the year 1909 and shortly afterwards malaria broke out there. This epidemic was authenticated by Breinl (1912) who gives an account of it. The record is of considerable value, because the epidemic is shown to be due beyond all doubt to malaria, and the conditions at Umbrawarra were, in all probability, identical with those obtaining on mining fields in earlier times where similar epidemics occurred. These rushes to new mineral discoveries attract men from other parts of the world, and all the mining camps of Northern Australia have contained men from New Guinea, which is a highly malarious country. In the case of Umbrawarra, Breinl traced the origin of the outbreak to miners from New Guinea arriving with parasites in their blood; from this fact it seems most likely that the earlier epidemics on other mining fields in the same regions of Australia were due to a like cause. The Umbrawarra epidemic came to an abrupt end, primarily by the departure of the majority of the miners, for in 1913, when the writer visited the field, there were only two miners remaining;

but it is strange that the disease did not spread to Pine Creek, a permanent settlement only thirteen miles distant and which during the height of the activities at Umbrawarra was in daily communication with it, receiving all the men who were seriously ill and many of whom must have had malaria parasites in their blood. The same species of Anopheline has been recorded from both places.

Breinl and Holmes (1915) visited several districts in the Northern Territory, including the Daly and Alligator Rivers, and Bathurst and Melville Islands; they found no signs of malaria among the natives in any of these localities either on blood examination or spleen palpation. In the same report it is mentioned that Holmes in 1912 found four out of twenty natives examined on Melville Island to be suffering from malignant tertian malaria, from which it is clear that within three years the disease had disappeared from the island without any special anti-malarial measures being taken.

The only other published records of malaria in the Northern Territory are those in the Annual Health Reports and in the Annual Reports of the Darwin Hospital. A brief outline of the local conditions will indicate that the figures in Table IV are only very approximate.

The total area of the Northern Territory of Australia is well over 500,000 square miles; there is only one* medical officer in the whole country and he spends practically the whole of his time in Darwin. For this reason most of the cases of sickness reported, and of deaths registered, are not certified by a qualified man, but are furnished by the local police; consequently the only reliable returns are those for the Darwin Hospital. In the year 1918 the highest population since 1910 was recorded, and this is given in Table III along with the latest figures available.

Table III.

Population of the Northern Territory.

Year	Europeans	Asiatics	Half-castes	Total
1918	3767	1177	118	5062
1921	2478	1094	?	3572

* From 1911 to 1915 there were four medical officers in the Northern Territory, and two of them did considerable travelling.

The aborigines consist of numerous small nomadic tribes, hence their numbers cannot be accurately determined. The most reliable estimate of the number of natives that the writer has ever been able to obtain, was given to him about ten years ago by an official who had spent over forty years in the country and who had travelled practically all over it. This officer was of the opinion that there were not more than 30,000 natives in the whole country. Although far from being precise these figures at any rate indicate that it is very thinly populated.

TABLE IV.

Malaria records for the Northern Territory

Year	Total Number of cases reported	Cases treated in Darwin Hospital	Deaths	Remarks
1897...	?	18	7	
1898...	?	8	8	
1899...	?	6	5	
1900...	?	5	6	
1901...	?	1	9	
1902...	?	1	6	
1903...	?	2	6	
1904...	?	12	8	
1905...	?	1	0	
1906...	?	6	0	
1907...	?	12	7	
1908...	?	23	16	
1909...	?	44	18	
1910...	?	27	18	
1911...	?	11	3	
1912...	?	12	0	
1913...	?	6	1	
1914...	1	—	0	
1915...	—	—	—	
1916...	'Prevalent'	?	15	For 18 months ending 30.6.1917
1917...	—	—	—	
1918...	45	?	?	
1919...	—	—	—	Not available
1920...	59	?	?	
1921...	'Many cases'	24	2	

In addition to the above table the following extracts from the Health Reports are appended.

1912. Malaria is not as prevalent as it is popularly supposed to be. The only death ascribed to malaria is registered 'kidney troubles and fever.' Practically all deaths outside Darwin are registered by the police, so the accuracy of the diagnosis is extremely doubtful. Malaria

is unknown at Pine Creek and Darwin, the two largest settlements. Some cases of malaria were found among the natives on Melville Island.

1913. The single death registered as due to malaria was diagnosed by a layman. Two of the medical officers travelled extensively during this year and only one case of malaria was seen, although this disease was specially looked for, and no cases were found on Melville Island where it was seen the year before.

1915-1917. Malaria was 'very prevalent' in several localities, e.g., the Pine Creek railway extension camps and Maranboy mining field. More than 50 per cent. of the cases were only diagnosed clinically, and although it is not stated, it is almost certain that a number of the cases were not seen at all by a medical man. It is considered that malaria is not endemic.

1918. One case was contracted in Darwin.

NOTE.—This is the only record of a case contracted in Darwin that the writer can find.

1920. All of the 59 cases reported for the year came from the country districts and were of a mild form. Three more serious cases were apparently contracted elsewhere.

1921. Many cases have occurred during the past few months, none of which were contracted in Darwin. The increase of the past few years is ascribed to the introduction of returned soldiers with parasites in their blood.

NEW SOUTH WALES

Early in the year Jamieson (1915) reported a case of malaria which the evidence showed to have been contracted at Gosford not far from Sydney. Commenting on this case, Cleland (1915) stated that apart from unreliable records in the comparatively early days of settlement he only knew of one other case contracted in the State. This was in a baby a few days after birth, who was born of a mother suffering from malaria at the time; he considers this to be a case of direct infection.

On the 17th March, 1915, 'Acute malaria' was made compulsorily notifiable throughout New South Wales; this regulation continued in force until 28th November, 1919, when it was withdrawn. The annual figures for this period are given in Table V.

Table V.

Cases of malaria notified in New South Wales.

Year	Number of cases
1915	105
1916	61
1917	17
1918	11
1919	35

It is not stated in these returns whether any of the cases were locally acquired, and all that can be gathered in this respect is that in 1915 the Chief Health Officer in his letter of presentation of the annual report states, that of the 105 cases recorded in that year, all except 14 were returned soldiers ; it is, of course, possible that all of the fourteen cases who were not soldiers also acquired their infections in other countries.

There are two other records of isolated cases which seem beyond doubt to have been contracted in New South Wales ; one of these was reported by Evans (1919) at Wyong, and the other by Clayton and Utz (1921) near Tumbarumba. This completes the published record of malaria for New South Wales.

VICTORIA

Doyle (1921) reported a case of malaria at St. Arnaud, which was locally acquired. As far as can be ascertained this is the only case of malaria ever recorded in Victoria.

In South Australia and Tasmania there is no evidence that malaria has ever occurred. The North-west of Western Australia which adjoins the Northern Territory is comparable to the latter both in its malaria incidence and conditions of living. No references to malaria in this part of the country can be found in the literature, nor are the Government reports from this State available, so actual figures cannot be given.

The writer had spent altogether upwards of five years in North Queensland and the Northern Territory (see map, places underlined), and during that time he has seen only two cases of malaria contracted in the country, the small outbreak on Palm Island in 1921 excepted. Experience has led him to the conclusion, that the inhabitants of Tropical

Australia are prone to ascribe all their ills to malaria and that this opinion is rarely confirmed by microscopic diagnosis.

The tendency of the layman to exaggerate the incidence of malaria reacts on the police, who in the absence of medical assistance are inclined to register all deaths not clearly due to violence as due to malaria. These figures are given in the annual health reports and so the popular and erroneous opinion of the prevalence of malaria is to some extent supported in official returns.

ANOPHELINE MOSQUITOES FOUND IN AUSTRALIA

According to Ferguson (1921) only five species of Anophelines have ever been recorded in Australia. They are :—

1. *A. corethroides*, Theobald, 1907.
2. *A.* (*Pyretophorus*) *atratipes*, Skuse, 1888.
3. *A.* (*Pyretophorus*) *stigmaticus*, Skuse, 1888.
4. *A.* (*Nyssorhynchus*) *annulipes*, Walker, 1850.*
5. *A.* (*Myzorhynchus*) *barbirostris*, de Wulp, var. *bancrofti*, Giles, 1902.

A. corethroides is only found in South Queensland.

A. atratipes was recorded by Bancroft (1908) from South Queensland and it was also recorded earlier by Skuse at Berowra, New South Wales.

A. stigmaticus has been recorded only once from a single locality in New South Wales.

These three species are only found in parts of Australia where malaria does not occur and are so restricted in distribution that they cannot have any bearing on the spread of this disease at the present time. The other two species, viz., *A. annulipes* and *A. bancrofti* are much more widely spread and as, from circumstantial evidence which is all that is available, they seem to be connected with malaria, their occurrence will be considered in more detail.

Distribution of *A. annulipes*. Hill (1922) summarises the distribution of this species as follows :—' *A. annulipes* is undoubtedly the most widely-distributed Anopheline found in Australia, having been recorded from Tasmania northwards to Banks Island (Torres Strait), and from South Australia, Central Australia, Northern Territory, South-west Australia, and North-west Australia. It is most probable that it does not occur in the elevated districts of South Australia and North Queensland (Atherton Tableland), and possibly not in some of the arid inland districts,

* Until recently, another species *Anopheles amictus* Edwards, 1921, has been confused with *A. annulipes*. *A. amictus* occurs at Townsville, Palm Island and Port Darwin. [Editors.]

Melville I.
AB
Bathurst I.
AB
Darwin
AB
Thursday I.
Banks I.
A
Papua
AB
Adelaide R. AB
Pine Creek A
Daly R.
A
Maranboy
AB
Roper R.
AB
Gulf of Carpentaria
Victoria R.
A
Borroloola
A
Cooktown
A
Port Douglas AB
Cairns B
Yarabah
Normanton
A
Croydon
Atherton
Einasleigh
Innisfail
Halifax AB
Ingham
Palm Is. A B
Townsville
Burketown
A
NORTHERN
TERRITORY
Camooweal
A
Lander Creek A
Georgina R.
Flinders R.
Charters Towers
Hughenden
Burdekin R.
B
A B
Bowen
Mackay
A
Rockhampton
A B
Gladstone
QUEENSLAND
WESTERN AUSTRALIA
SOUTH AUSTRALIA
Bundaberg
Maryborough
Gympie A
Caboolture
B
Enoggera
Brisbane
A
NEW SOUTH WALES
Perth A
Wyong
A
Gosford
A
Newcastle
Sydney
Adelaide
A
Tumbarumba
Murray R.
A
St. Arnaud
A
VICTORIA
Melbourne
A
500 miles (English)

although its presence in such localities as Wire Creek Bore and Lander Creek (Central Australia), indicates that only the absence of suitable breeding places would inhibit its existence in the latter.'

Distribution of *A. bancrofti*. This species is not so widely spread as *A. annulipes*. Hill states that it is found only in the coastal districts of Queensland, some of the Torres Straits Islands, and the ' . . . Northern (coastal) districts of the Northern Territory . . .' but one of the localities given by Hill, viz., Horseshoe Creek is over 150 miles from the coast. As it is possible that this species has more to do with malaria in Australia than has hitherto been supposed, it is proposed to consider the records of its occurrence in more detail. These are summarised in Table VI.

TABLE VI.

Principal records of the occurrence of *A. bancrofti* in Australia.

Locality	By whom recorded	Remarks
Brisbane	Bancroft (1908)	Found in the scrub from Enoggera to Caboolture; males, larvae, and eggs never found.
Brisbane	Cooling (1913)	Once taken in a house; a few females taken in the scrub; no larvae found.
Brisbane	Cooling (1914)	Four specimens taken for the whole year.
Rockhampton	Taylor (1916)	Numerous adults, no larvae.
Burdekin River	Taylor (1913)	
Townsville	Taylor (1913)	Hill (1922), writes that he has failed to find the species over a period of 2¼ years' continuous observation and concludes that it has died out.
Halifax	Taylor (1916)	
Palm Islands	Hill (1922)	Plentiful within 200 yards of No 2 aboriginal camp and close behind main camp. *A. annulipes* not taken nearer than ¼ mile of camp clearing.
Cairns	Taylor (1916)	
	Taylor and Breinl (1918) ...	Numerous, and breeding freely in swamps in and around town.
Northern Territory ...	Hill (1922)	Twelve distinct localities given, including Melville and Bathurst Island (see map).
		Note.—The mosquito observations in the N.T. are more thorough than in any part of Tropical Australia except for a small area round Townsville.

With the exception of Brisbane, Townsville, Cairns and to some extent the Northern Territory, the records in Table VI refer to a single observation. It is probable that more extended work would reveal the presence of *A. bancrofti* in many other parts of Northern Australia, but it is unlikely that this species exists far south of Brisbane, because it has never been recorded in New South Wales at all, and the knowledge of mosquitoes in this State is much more advanced than it is in Queensland. In fact, as far as the records go, there is some evidence that A. *bancrofti* is not found in any numbers south of about 19° South Latitude.

THE INSECT VECTOR OF MALARIA IN AUSTRALIA

The Medical Journal of Australia has on more than one occasion (Leading articles, 1915, p. 171 and 1921, p. 512, etc.), pointed out that the mosquito carrier of malaria has not yet been determined. Breinl (1912) stated that *A. annulipes* was the probable vector at Umbrawarra ; in support of this he quotes the successful experiment of Kinoshita (1906), who successfully infected *A. annulipes* with *Plasmodium falciparum* in Formosa. A little later Breinl (1914) definitely stated that *A. annulipes* was the carrier of malaria in Australia, but quoted no authority. Since these two references *A. annulipes* has been frequently mentioned in the medical literature of Australia, being variously described as ' the probable carrier,' ' the presumed carrier,' ' the carrier,' etc., but in most cases no authority is given, and when it is, Kinoshita (1906) is the only reference. The statement by Harrison (1922) is an accurate summary of the present state of our knowledge in this respect, when he says that there is evidence that the local Anophelines are capable of acting as intermediate hosts for malaria parasites.

The only record of *A. annulipes* as a malaria carrier given by Chanal (1921), is the single experimental result obtained by Kinoshita already referred to ; Chanal's conclusion is that *A. annulipes* should be classed as dangerous. But it should be noted that although Kinoshita states that he infected 60 per cent. of his mosquitoes, a detailed study of his experiments shows that he used the species on three occasions. The first time, out of five fed, all died within three days, the second time nine insects were used and all died within three days of feeding, the third time eight mosquitoes were used, of which three died within three days

and three of the remaining five became infected. It is this result Kinoshita gives as 60 per cent. positive ; only *P. falciparum* was used.

A. bancrofti seems by common consent to have been almost completely ignored as a possible malaria carrier in Australia, for the only references to this species in this connection are the following, viz. :—Cleland (1910) includes it in a list of the then known malaria carriers. It is next mentioned by Cooling (1914) who suggests that it may be a malaria carrier, because Stephens and Christophers (1902) were successful in infecting *A. barbirostris* in the laboratory in India. Since that date, according to Chanal (1921), *A. barbirostris* has been found in nature and infected in the laboratory both with *P. falciparum* and *P. vivax* on several occasions in various parts of the Malay Archipelago ; but as all these records refer to a different variety of the species which does not occur in Australia, they have no bearing on the subject. One other reference to *A. bancrofti* as a malaria carrier is made by Breinl (1915) who, in an article on New Guinea, states that *Nyssorhynchus bancrofti** is not a malaria carrier. This statement is not supported by any evidence.

With regard to the distribution of Anophelines in Australia, Breinl (1914) says, '. . . The distribution of malaria in Australia corresponds, on the whole, with the incidence of the mosquito *Nyssorhynchus annulipes* . . . It is curious to note that there are localities where the mosquito has been found, but where malaria is practically non-existent.'

Again, Breinl and Taylor (1918) remark, '. . . *Nyssorhynchus annulipes* which, judged by its distribution in relation to malarial infested regions in Northern Australia, most probably acts as a malaria carrier . . .'

These two statements may be more or less correct as far as they go, but they do not explain why malaria is practically never found far south of Cairns, whereas *A. annulipes* is spread all over Australia. If the explanation of the restriction of malaria to Northern Australia is to be found in the distribution of a special Anopheline, it will be found that the occurrence of *A. bancrofti* much more nearly corresponds with the malaria distribution than does *A. annulipes*. *A. bancrofti*, however, also exhibits one or two striking exceptions to the rule, so it is considered that the peculiar distribution of malaria in Australia is due to other causes, not yet ascertained.

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* *Myzorhynchus bancrofti* is obviously the species intended, for Taylor (1914) in the list of mosquitoes taken by Breinl on this expedition includes it, and as far as the writer can ascertain there is no such species as *Nyssorhynchus bancrofti*.

As far as the writer can find out, the only explanation that has ever been offered as to why malaria fails to become established in the greater part of Australia is the mathematical hypothesis of Ross (1910) ; all the authors who mention this subject are of the opinion that there are too few mosquitoes or too few susceptible human beings in most parts of Australia. It is unlikely this is the sole reason, if it is the reason even in part, for it is by no means in the most thickly populated parts of Australia where Anopheline mosquitoes are found that malaria outbreaks occur.

There is another set of conditions which seem to the writer worthy of consideration, and which have never been considered, and that is the relation between malaria outbreaks and meteorological records. Gill (1920 and 1921a) has studied the incidence of malaria in parts of India along with the mean temperature and relative humidity readings, and the same author (1921b) extended his observations to England. As a result of this work he considers it probable, that before malaria is able to spread in a locality it is necessary to have a monthly minimum mean temperature of 61° F. and a minimum mean relative humidity of 63 per cent. At the same time he points out this is not yet conclusively proved. In this connection it is worth noting that in Kinoshita's successful experiment with *A. annulipes* the temperature remained between 28° and 30° C. the whole time, and in his conclusion he states that complete development of the oocysts of *P. falciparum* cannot take place in this mosquito except in a high and unvarying temperature.

SUMMARY

As far as can be gathered from the incomplete and unreliable records available, malaria is only mildly endemic in Australia north of 19° South Latitude. *A. annulipes* and *A. bancrofti* the only two possible malaria carriers in Australia under present conditions are much more widely distributed than is malaria.

In various localities north of 19° South Latitude small epidemics of malaria occur from time to time ; these outbreaks are of short duration, their origin is generally traceable to the introduction of malaria carriers from abroad, the disease does not spread to adjoining camps and towns, and soon dies out, without any very active anti-malarial measures being instituted.

The scarcity of population and Anopheline mosquitoes is not a satisfactory explanation of the absence of malaria from the greater part of Australia.

It is of the first importance to discover the mosquito carriers of malaria in Australia, and when this has been done, work along the lines of Gill in India and England would possibly yield interesting and valuable results.

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COCCIDIOSIS OF CATS AND DOGS AND THE STATUS OF THE *ISOSPORA* OF MAN

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PLATES IX-XIV.

Our knowledge of the coccidia of dogs and cats commences with certain observations recorded by Finck (1854) on the changes undergone by the intestinal epithelium of cats during the process of food absorption. From that time to the present day it has been generally assumed that these animals harbour only one coccidium, which has been usually described during recent years under the name *Isospora bigemina* (Stiles, 1891). Though the measurements of the oocysts given by various observers who have studied the coccidia of these animals have differed considerably, the view that only one form exists has been rigidly adhered to with few exceptions. Perroncito (1882) appears to be the first to have considered it possible that more than one form occurred in these animals, for he separates the one described by Grassi (1879) from that recorded by Rivolta (1874-1878), while Neumann (1888), Railliet (1895) and Neveu-Lemaire (1912) seem to have held the same view. Dobell (1919, p. 177) states that there is no really conclusive evidence to prove that the *Isospora* of the cat is the same as that of the dog, or that both are merely varieties of one species, but he refers to all the coccidia of these animals by the name *Isospora bigemina*, pointing out, however, that Grassi's name *Coccidium Rivolta* has priority over that of Stiles. Reichenow (1921) definitely asserts that the form in the dog is probably distinct from that in the cat, while Nöller (1921), without giving any details, writes of a small and a large form in the cat.

Observations which have been made by the writer during the

past twelve months reveal the fact that there are at least three species of *Isospora* in these animals in England. One of these has an oocyst about 12-15 microns in length, another an oocyst about 25-30 microns in length, and a third an oocyst about 40-45 microns in length. The text-fig. 1 shows the relative size and appearance of the three types compared with the one discovered in man during the war. If these dimensions are kept in mind, the different accounts which have been given by various observers become at once intelligible, and it is possible to identify with some degree of certainty which form was actually under observation. All three have been previously recorded in the literature. In addition to the species of *Isospora*, dogs harbour an *Eimeria* with which we are not for the moment concerned.

HISTORICAL REVIEW OF LITERATURE

The first description of a coccidium of the cat was published by Finck (1854). His paper is difficult to obtain, but fortunately Davaine (1860) quotes in full the passage dealing with the bodies observed by this author. As it is of such importance from the present point of view it is quoted *in extenso* from Davaine, pp. 259-260.

"Sur le même animal (le chat) nous avons rencontré une autre forme bien plus singulière (fig. 22). Beaucoup de villosités, semblables du reste à celles chargées de graisse, à la place de gouttes graisseuses, renfermaient, en quantité considérable, des *corpuscules* que nous appellerons *géminés*, parce que le plus souvent ils étaient réunis par paires. Tantôt une seule et même villosité offrait à la fois et des gouttes huileuses manifestes et des *corpuscules géminés*, le tout entremêlé d'une manière irrégulière ; tantôt les *corpuscules géminés* remplissaient seuls le bout de la villosité. Ils étaient pour la plupart elliptiques, et leur grand diamètre atteignait à peine un centième de millimètre ; la plupart mesuraient O^{mm}, 08 sur O^{mm}, 07, ou bien O^{mm}, 1 sur O^{mm}, 09. Leur contour était fin, net, très noir ; leur contenu variable, occupant tantôt presque toute la cellule, plus souvent accumulé vers son centre. C'était une matière granuleuse réunie en une ou plusieurs masses. Il nous a semblé parfois voir une enveloppe commune pour deux corps géminés.

"Quel est la nature de ces corps ? Remak représente un corpuscule semblable au premier aspect, seulement plus grand et non *géminé*. Il croit devoir le considérer comme un parasite particulier qui se développerait dans les cylindres épithéliaux des glandes de Lieberkühn et dans ceux des conduits biliaires. Il cite Hake et Nasse comme ayant trouvé des formes semblables, par masses, dans le foie du lapin. Kölliker a observé la même chose. Selon lui, les corpuscules du foie du lapin seraient des oeufs de bothriocéphale ; ceux des villosités du même animal, plus petits que les premiers, des oeufs d'entozoaires, siégeant dans l'intérieur des villosités et peut-être aussi dans les cellules épithéliales distendues. Dans ce

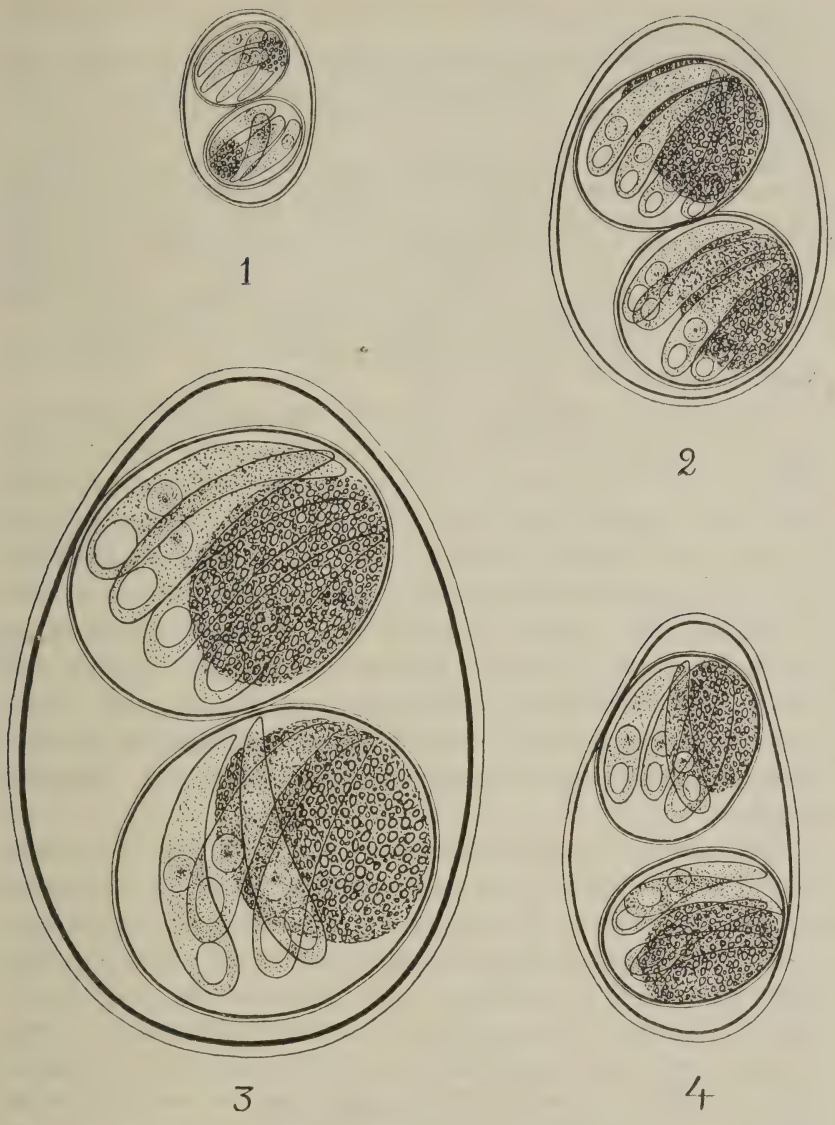

FIG. 1. Diagram of the oocysts of the *Isospora* of cats, dogs and men. × 2000.

1. Oocyst of the small form which occurs in the deeper tissues of the villi of cats and dogs and man (*Isospora bigemina* and *Isospora hominis*).
2. Oocyst of the intermediate sized form which occurs in the epithelium of the villi of cats and dogs (*Isospora rivolta*).
3. Oocyst of the large form which occurs in the epithelium of the villi of cats and dogs (*Isospora felis*).
4. Oocyst of the large form which probably occurs in the epithelium of the villi of man (*Isospora belli*).

dernier cas, ils ressemblent, selon lui, à des grosses gouttes graisseuses remplissant les cellules épithéliales.

"Nous n'avons rien trouvé de pareil dans les cellules épithéliales de notre chat ; mais son foie renfermait des amas d'entozoaires plats, elliptiques, long d'un millimètre, probablement des douves. Ils étaient contenus dans des espèces de kystes.

"Quant à nous, tenant compte de l'énorme quantité des corpuscules en question, de l'absence de toute forme semblable dans la cavité de l'intestin, de leur absence dans toutes les villosités n'ayant point subi l'espèce de macération caractérisant les villosités farcies de globules graisseux, enfin de certaines formes de transition entre ces derniers et les *globules géminés*, nous croyons ne pas trop nous hasarder en rattachant les corpuscules en question au fait du méchanisme de l'absorption graisseuse. C'est tout ce que nous pouvons en dire quant à présent."

(Henri Finck : *Sur la physiologie de l'épithélium intestinal.* Thèse de Strasbourg, 1854, 2e série, n° 324, p. 17).

The important points to note from the above description are these. The sporocysts or *corpuscules géminés*, as Finck styled them, occurred in the substance of the villi and not in the epithelium of the cat's intestine. They measured 8 by 7 microns up to 10 by 9 microns, had definite contours, and were sometimes enclosed in pairs in a common membrane which was evidently the oocyst wall. As pointed out by Railliet and Lucet (1891), Finck's measurements have been wrongly quoted as ten times higher than they actually were by several observers, as, for instance, Pfeiffer (1890, 1891), Neumann (1892, p. 467). Dobell (1919) inadvertently refers to Finck's investigations as having been made on the dog, instead of the cat.

The reference to the similar but larger bodies seen by Remak, referred to by Finck, have to do with a paper by this author published in 1845 on the occurrence of what were evidently the oocysts of a coccidium in the intestinal wall of the rabbit. Vulpian (1858) cites Finck's observations, but it is not quite clear that he actually observed the oocysts of the cat coccidium himself. Rivolta (1873, p. 382), referring to the presence of psorosperms in domestic animals, says that they had previously been observed by Finck (1854), and also by Ercolani in 1859, in the cat. Perroncito (1882) also quotes Ercolani as having made this observation. Virchow (1860, p. 342 and p. 527) was the next observer to give any details of their structure, though, like Finck, he regarded them as products of fat absorption. He noted that the villi of the greater part of the intestine of a dog were infiltrated with psorosperms. They were on the surface of the intestine, but a larger number were free in the

intestinal contents. They occurred in the interior of the villi and were relatively small and regularly arranged in pairs enclosed by a double contoured membrane. He says they were evidently similar to the paired bodies described by Finck from the cat. He records and figures the oocysts of a coccidium which he found in the kidney of a bat, and which he regarded as similar to the one seen by him in the dog. The parasite of the bat evidently belongs to the genus *Isospora.*

Leuckart (1860, p. 11, and 1866, p. 21) mentions the fact that the intestinal mucosa of a dog which had been used for experiments with *Trichina* was much altered, and covered with a layer of small, egg-shaped psorosperms. He gives no details of their size or structure. He again (1863) refers to them, but is inclined to regard them as metamorphosis products in the intestinal wall. Another reference to these bodies found in another dog by the same author (1879, p. 282) gives no further information, but he was aware of Finck's work and evidently regarded the structures he had encountered as the same as those seen by Finck. He now describes the condition as due to an accumulation of parasites in the villi.

Rivolta (1874) gave a description of certain oviform cells (*cellule oviformi*) which he had found in the intestinal villi of dogs and cats. In his account, which deals entirely with those seen in dogs, he says they had walls showing a double contour, and varied in length from 8 to 12 or even 15 microns; while in breadth they measured 8 microns. The contents of some of the oviform cells are described as being granular and in the form of a nucleus, or as an elongated body like an embryo with granular material at its centre. In some, however, it is stated that in addition to a granular nucleus there were distinctly three or four elongated corpuscles somewhat irregular in shape. There are four figures accompanying this description, and two of these show quite clearly the granular mass and four small ovoid bodies. The oviform cells are described as occurring in the tissues of the villi especially near their tips and not in the epithelium. As evidence of this, a case is quoted where they were present in the villi of a dead animal which, owing to cadaveric changes, had lost its intestinal epithelium entirely. These oviform cells are again described by Rivolta (1877). In this paper mention is made of Finck's observations, and it is pointed out that invasion

by the cells produces grave alterations in the structure of the villi. In a further communication, Rivolta (1877a) states that he has found other cases of the infection in dogs. Examination of the oviform cells in Müller's solution showed that they constantly contained four long corpuscles with rounded ends. Two other stages are described and figured showing the bodies filled with a granular mass which may have indications of a central constriction. He ventures the suggestion that proliferation into two is taking place. The figures show this clearly. The length of the bodies is given as 13 to 16 microns, and the breadth as 12 microns. The statement is made that they are identical with the *corpuscules géminés* observed by Finck. Rivolta compares them with the psorosperms of the liver of the rabbit, and points out that they differ from these psorosperms in that they do not occur in the epithelium, and that segmentation takes place in the body of the host. He sums up his description by stating that there occur two types of these oviform cells. In one type the contents consist of a nucleus with four elongate corpuscles, while in the other there is a large granular nucleus which at times is in process of segmentation. In a later paper, Rivolta (1878) attempts to classify the psorosperms and gregarines of animals. He names the oviform cells of the dog and cat *Cytospermium villorum intestinalium canis*. He again states that two types of this parasite occur. The first varied in length from 8 to 12 microns, and had a breadth of 8 microns. Within was a single elongate granular body like an embryo. After a few days in water there developed three corpuscles and a granular nucleus. The second type was larger, and varied in length from 12 to 16 microns, and had a breadth of about 12 microns. The contents consisted of a single large granular mass which sometimes showed signs of segmentation.

From the above summary of Rivolta's descriptions it is clear that the larger type is the oocyst and the smaller one the sporocyst. He correctly observed the division of the granular mass into two sporoblasts, but did not realise that each of these gave rise to one of the smaller types which are sporocysts. It is evident that the wall of the oocyst was not very resistant, and easily liberated the sporocysts. In his earlier papers he correctly noted and figured the four sporozoites and the residual body within the sporocysts. It

is evident that the infection was limited to the internal tissues of the villi, and did not occur in the epithelium. The size of the oocyst was 12 to 16 microns by 12 microns, and that of the sporocyst 8 to 12 microns by 8 microns. The development was often completed before the oocysts had left the tissues. Incompletely developed sporocysts continued their development in water.

In his book, already noted above, Leuckart (1879, p. 282) discusses the changes produced in the intestinal wall by coccidia generally. He says he has seen these parasites in both dogs and cats. In the latter animals he states that they occur in the epithelium, where complete development takes place. In the case of the dog, they were in the villi, and he evidently regarded them as similar to the structures seen in this situation by Finck, but is doubtful about those described by Rivolta. As regards the cat, Leuckart is the only observer to refer to the complete development of the oocyst in the epithelium. Finck and Rivolta, together with Railliet and Lucet and Stiles, whose observations are considered below, all state that this takes place in the deeper tissues of the villi. Leuckart's account is not always clear as to the actual animal he is referring to, but the statement quoted definitely refers to the cat. As will be explained below, the oocysts of coccidia which develop in the epithelium do not commence to develop till they have left the body, so that Leuckart's statement is difficult to understand. It is possible that oocysts of the larger forms in the epithelium might develop in animals which had been dead for some considerable time, or that Leuckart actually observed the oocysts of the small form in an unusual situation in the epithelium. On the other hand, he may have seen both a large and a small form in these animals, and confused the two. It seems impossible to be certain of the form he refers to in the cat, but his statements about the one in the dog are much more precise.

The next observer to make a contribution to the subject from personal observations was Grassi (1879), who gives a brief account of a coccidium which he calls *Coccidium Rivolta*, from the intestine of the cat. The oocyst is described as giving rise to two spores, each of which contains four germs. In later papers (1882, 1883) he gives under the same name a more detailed description. The oocyst is said to be elliptical in shape with one end more pointed

than the other. At the pointed end there could be detected a sort of spiracle or micropyle. The measurements of the oocyst are given as 30·8 to 27 microns by 24 to 22 microns. Within it is a sphere varying in diameter from 10 to 20 microns, with a central clear area or nucleus. The sphere divides into two daughter spheres, each having a diameter of 14·3 microns. Two sporocysts result, within which are found four embryos and a large residual body. It is important to note that the parasite is described as occurring in the epithelium of the intestine. The description is accompanied by figures which illustrate clearly the structure of the oocysts. From Grassi's account there can be no doubt that he was dealing with a coccidium which was entirely distinct from that described by Finck, Virchow and Rivolta. As pointed out above, this distinction was recognised by Perroncito (1882) and others.

Pachinger (1887) states that he had seen a sporozoon in the oesophagus, stomach and whole length of the intestine of the domestic cat, and that he had encountered a similar form in the kidney of the dog. He says that it belonged to the monospore coccidia with four sickle-shaped bodies. It is probable he was observing the sporocysts of an *Isospora* of the cat, but there are no means of identifying it with certainty, as no measurements were given. The structures he records from the kidney of the dog are quite unidentifiable.

Railliet and Lucet (1888) published an account of oviform bodies which they found in the villi of a dog. They noted their occurrence in pairs, and remarked on their resemblance to the bodies described by Virchow and Rivolta. On account of their association in pairs, they hesitated to pronounce an opinion as to their coccidial nature. After further study, Railliet and Lucet (1890) gave a brief but clear description of these bodies as coccidia which they had observed in the pole cat as well as the dog. In the dog the oocysts are said to vary in length from 12 to 15 microns, and in breadth from 7 to 9 microns. The contents of each divide into two masses, and each of these gives rise to four spores. The fully developed oocysts may occur in the fresh villi, but usually complete development does not take place till they have been in water for a few days. The similar form with oocyst, measuring 8 to 12 microns by 6 to 8 microns, discovered in the pole cat (*Mustela putorius*) occurs in the deeper tissues of the villus.

Stiles (1891) refers to the work of Railliet and Lucet, and says that he has seen the cysts in the villi of dogs. He noted that each might contain a single large mass of cytoplasm or two separate masses suggesting a division into two of the large mass. Stiles gives the name *Coccidium bigeminum* to this parasite. Railliet and Lucet (1891), in a further communication on the subject, accept the name *Coccidium bigeminum* given by Stiles. They refer to the work of Rivolta and Finck, and say there is no doubt that these observers had studied the same organism. They now describe three varieties of the parasite as occurring in the dog, cat and pole cat, which they regard as varieties of *Coccidium bigeminum* owing to differences in the size of the oocysts :—

Coccidium bigeminum var. *canis* 12—15 × 7—9 microns.
Coccidium bigeminum var. *cati* 8—10 × 7—9 „
Coccidium bigeminum var. *putori* 8—12 × 6—8 „

As pointed out by Wasielewski (1904) these variations in size are insufficient to justify a separation of varieties on this basis alone.

The papers by Railliet and Lucet are not illustrated, but a figure by Railliet appears in the English translation of Neumann's work on 'Animal Parasites' (1892, p. 437). This figure again appears in the second edition of the *Traité de Zoologie Médicale et Agricole* by Railliet (1895, p. 145). Stiles (1892) gave a fuller and illustrated account of the *Coccidium bigeminum* of the dog. He described the development of the oocyst with the production of two sporoblasts and two sporocysts, and the formation within each sporocyst of four sporozoites and a residual body. A figure of a section of the villus shows the presence of oocysts containing the sporoblasts or undeveloped sporocysts within the deep tissues of the villus. The size of the oocyst is given as 14 by 8 microns.

From the description of Railliet and Lucet, and Stiles, it is evident that they were dealing with the coccidium seen by Finck, Virchow and Rivolta in dogs and cats. These observations appear to be the last ones which have been made on the small *Isospora* of these animals.

Wasielewski (1904) gave a detailed account illustrated with excellent microphotographs of the oocysts of a coccidium, called by him *Diplospora bigemina*, which he had observed in cats that were used for experiments on amoebic dysentery by Jürgens. The oocysts which he observed varied in size, and he gives a series of

measurements in microns as follows:—22 by 19, 25 by 20, 25 by 22, 35 by 23, 35 by 25, 35 by 27, 38 by 32, 40 by 28. He describes the development of the oocyst in detail. The contents contract to form a sphere, which has a diameter of 18 to 25 microns according to the size of the cyst. Two daughter spheres are formed by division of the large sphere, and these vary in diameter from 16 to 18 microns in the larger oocysts and from 11 to 12 microns in the smaller ones. The daughter spheres become sporocysts, within each of which are developed four sporozoites 11 to 12 microns in length and a residual body 6 to 8 microns in diameter. The earlier stages of the parasite were found only in the epithelium of the small intestine and never in the submucosa, so that Wasielewski considered that the statements which had been made of a coccidium limited to the submucosa required some qualification. Schizonts in the epithelium and motile merozoites free in the lumen of the intestine were also seen.

This coccidium is clearly distinct from that studied by Finck, Virchow, Rivolta, Railliet and Lucet, and Stiles. From the size of the oocysts they appear to fall into two categories, as noted by Reichenow (1921), the one with oocysts measuring 22-25 by 19-22 microns and the other with oocysts measuring 35-40 by 23-32 microns. Those of the first category clearly correspond with the parasite described by Grassi (1879, 1882, 1883). Wasielewski also gave measurements of 18 by 25 microns for the oocysts and 11 by 15 microns for the sporocysts of a form seen by him in the dog. He regarded it as *Coccidium bigeminum*, but it corresponds exactly with Grassi's *Coccidium Rivolta*.

Basset (1909) without giving any description of the parasites, discusses the pathogenic effect of coccidia, which he calls *Diplospora bigemina*, in young dogs. He also records a round coccidium, 14 microns in diameter, as occurring in dogs and ferrets, but there is no evidence that these were actually coccidia, as no mention is made of any development.

Swellengrebel (1914) gave a complete account of the development of a coccidium of the cat under the name *Isospora bigemina*, which appears to be identical with the large form noted by Wasielewski. He described for the first time the process of schizogony in the epithelial cells of the small intestine, the evolution of the macrogametocytes and microgametocytes, and formation and

development of the oocysts. The measurements of the oocyst are given as 39 to 47 microns by 26 to 37 microns. The sporocysts vary in length from 21 to 24 microns, and in breadth from 18 to 19 microns. Within the sporocyst there are formed four sporozoites measuring 18 by 4 microns, and a large residual body. Swellengrebel clearly states that the appearances are absolutely unlike those figured by Stiles, but hesitates to establish a new species.

Weidman (1915) described a coccidium, which he called *Coccidium bigeminum*, in 'swift foxes' in the Western United States. The oocysts varied from 25 to 40 microns in length by 25 to 30 microns in breadth. The sporocysts measured 16 to 20 by 14 to 18 microns. Owing to the difference in dimensions from the form described by Railliet and Lucet, and Stiles, Weidman suggests the 'new varietal name "canivecolis".' He gives figures of the oocyst containing two sporocysts, with four sporozoites and a residual body. Mesnil (1916) states that Weidman regarded it as a variety *canivecolis* of *Isospora bigemina*.

Wenyon and O'Connor (1917) found an *Isospora* of the cat very common in Alexandria, and Dobell (1919) records a similar experience in England in the case of cats used for experiments on amoebic dysentery. In both these instances the oocysts were of the large type. This was also the writer's experience during experiments on cats conducted in London in 1912.

Hall (1917) discovered a coccidium in dogs in Detroit. On account of its large size, he thought it was different from *Isospora bigemina*, but later Hall and Wigdor (1918) concluded that it was a larger form of the same parasite, and wrote of it as *Diplospora bigemina*. The oocysts measured 36 to 40 microns in length by 28 to 32 microns in breadth. The sporocysts had a diameter of 10 to 20 microns, and the sporozoites measured 12 by 4 microns. Oocysts of these dimensions occurred in the majority of dogs, but in one animal a smaller strain was seen, the oocysts measuring 20 by 18 microns and the sporocysts 12 by 11 microns, with sporozoites 10 microns in length by 3 microns in breadth. They state that this distinction in the size was quite marked, and that it raises the question as to whether the small one should be regarded as a variety or species. They go on to say that it is possible that there are several species of *Diplospora* in the dog

characterised by considerable difference in size. The length of time required for the development of the oocyst of the larger form was two days when kept in 10 per cent. potassium bichromate solution. Under other conditions, which they state more nearly resemble those of nature, the time required may be two weeks or longer.

Reichenow (1921), referring to the *Isospora* of cats and dogs, expresses it as his opinion that Wasielewski was probably dealing with a mixed infection of two distinct coccidia in the cats he examined. He also states that the form he had observed in the dog in Germany differs from that in the cat, and resembles the one with smaller oocysts studied by Wasielewski. For the oocysts of the dog form he gives a length of 21 to 24 microns, and a breadth of 18 to 20 microns. The sporocysts, which are oval in outline, measure 14 to 16 microns by 9 to 10 microns. Nöller (1921), in a brief reference to the coccidium of dogs and cats, refers to the large and small form in cats and the one in dogs. He has been able to infect young dogs in series with the oocysts. No details of the dimensions are given. Marotel (1922) studied the *Isospora* of the cat. His measurements are as follows:—

Oocysts 45—48 × 34—36 microns.
Sporocysts 22—24 × 17—19 microns.
Sporozoites 18—20 × 4—5 microns, residual body in sporocyst 10—12 microns.

He proposes to call the coccidium *Isospora cati*. In order to facilitate the following discussion, the various dimensions in microns given by the above observers for the oocysts and sporocysts of the dog and cat parasites are arranged in tabular form:—

Table I.

	Oocyst	Sporocyst
Finck (cat)	...	8 — 10 × 7 — 9
Virchow (dog)	Like those described by Finck	...
Rivolta (dog and cat)	12 — 16 × 12	8 — 12 × 8
Grassi (cat)	27 — 30·8 × 22 — 24	14·3
Railliet and Lucet (dog)	12 — 15 × 7 — 9	...
——— (cat)	8 — 10 × 7 — 9	...
——— (pole cat)	8 — 12 × 6 — 8	...
Stiles (dog)	13·6 — 15·9 × 7·9 — 9·9	...
Wasielewski (cat)	35 — 40 × 23 — 32	16 — 18
——— (cat)	22 — 25 × 19 — 22	10 — 12
——— (dog)	18 × 25	11 — 15
Swellengrebel (cat)	39 — 47 × 26 — 37	21 — 24 × 18 — 19
Hall and Wigdor (dog)	36 — 40 × 28 — 32	10 — 20
——— (dog)	20 × 18	12 × 11
Reichenow (dog)	21 — 24 × 18 — 20	14 — 16 × 9 — 10
Marotel (cat)	45 — 48 × 34 — 36	22 — 24 × 17 — 19

From the above table it will readily be seen that the oocysts described fall into three groups.

(1) There are the small forms described by Finck, Rivolta, Railliet and Lucet, and Stiles. Finck did not state the actual measurements of the oocysts, but from the size given for the sporocysts and the fact that in his description he says that two of these sometimes occur together enclosed by a common membrane, it is safe to assume that the oocyst would have dimensions similar to those described by Rivolta, Railliet and Lucet, and Stiles. The forms seen by Virchow are evidently similar, for he says they occur in the interior of the villi of dogs, are relatively small and regularly arranged in pairs enclosed by a thick, double contoured membrane. It is probable also that those described by Leuckart are of the same type.

(2) The second type has an oocyst of intermediate size. This was first seen by Grassi in the cat, later by Wasielewski in the cat and dog, by Hall and Wigdor in the dog, by Reichenow in the same animal, and possibly by Nöller in the cat and dog.

(3) The third type has an oocyst of much larger size. This was first definitely described by Wasielewski and Swellengrebel in the cat, and was seen by Wenyon and O'Connor, Dobell, Hall and Wigdor, and Marotel.

That these three types represent distinct species seems clear from the above records, and from observations to be recorded in this paper. In a recent study of English cats, the oocysts which occurred in the faeces were uniformly of large size, while those which were found in dogs' faeces were of the intermediate type. In one instance only was an infection of the cat with the small type seen. In this case the large form occurred also and it was clearly evident that the small one was limited to the deeper tissues of the villus, while the large one developed in the epithelium. Furthermore, development of many of the small oocysts was completed in the tissues of the villi, while those of the large form did not take place for some days after it had left the body. That the oocysts of the smallest form sometimes escape in the faeces in the undeveloped condition is demonstrated by an observation which has just been made by Mr. Leslie Sheather, of the Royal Veterinary College, with whom the writer has discussed his investigations on coccidiosis of dogs and

cats. By a process of concentration employed for the detection of worms' eggs in faeces, Mr. Leslie Sheather discovered that one dog was infected with the small form and another with that of intermediate size. The small oocysts measured about 12 microns in longest diameter, and like those of intermediate size were in the undeveloped condition. They proceeded to development when kept outside the body.

NOMENCLATURE

As regards the nomenclature of these parasites, there appears to be no great difficulty, though the name *Coccidium bigeminum* Stiles, 1891, has been employed indiscriminately for all three forms. Apart from Rivolta's name *Cytospermium villorum intestinalium canis* which he proposed in 1878, Grassi's name *Coccidium Rivolta* (1879) is the first one to be given to any one of these Coccidia. As pointed out above, Grassi was dealing with the oocysts of intermediate size in the cat, and, assuming that this form is the same as that of corresponding size from the dog, his name has priority. The name of this coccidium is, therefore, *Isospora rivolta* (Grassi, 1879). For the small form in the dog and cat the correct name is *Isospora bigemina* (Stiles, 1891). This leaves the large form in the dog and cat still unnamed, for the name *Isospora cati* suggested by Marotel (1922) cannot stand, as Railliet and Lucet (1891) employed the name *Coccidium bigeminum* var. *cati* for the small form in the cat, which if recognised as a distinct species from that in the dog, would become *Isospora cati.* For the large species the name *Isospora felis* is suggested. There are thus to be distinguished in dog and cat three species of *Isospora*:—

Isospora bigemina (Stiles, 1891).
Isospora rivolta (Grassi, 1879).
Isospora felis n. sp.

It is assumed that these different parasites are able to infect both dogs and cats, but it is possible that each animal has its own species. This can only be determined by more detailed observation and cross-infection experiments with clean animals. Railliet and Lucet (1891) have stated that the small forms in the cat, dog and pole cat are varieties of *Isospora bigemina*, while Weidman (1915) has made a similar suggestion for the large coccidium described by him

in the fox. His name was not properly proposed, as he merely says he advances a new varietal name 'canivecolis.' Mesnil (1916), in a summary of Weidman's paper, writes the specific name *canivecolis*, while Hall and Wigdor (1918) give it in full *C. bigeminum canivecolis*. This parasite, which is certainly not a variety of *Isospora bigemina*, may be identical with *Isospora felis*, but on the other hand it may be distinct. In the latter case the name *Isospora canivecolis* would be correct.

There exists also in dogs in England an intestinal *Eimeria* as recorded by Brown and Stammers (1922). For this parasite the name *Eimeria canis* is proposed.

Though Grassi (1879) proposed the name *Coccidium Rivolta* for the parasite he found in the cat, this name has been modified by several observers, in spite of the fact that Grassi repeated the name in his later papers (1882, 1883). Dobell (1919) in discussing this question, says that it is his view that Grassi's name should be changed by putting '*rivolta*' in the genitive, in which case the name would be *Isospora rivoltae*. He thinks that a form such as '*rivoltai*' is objectionable. There seems, however, to be no real reason why the name should be changed at all, and to keep it in the form proposed by Grassi is in accordance with Rules of Nomenclature. Both the changes discussed by Dobell have, however, been previously made. Thus in the English translation of Leuckart's work (1886) there appears a note on page 221 initialed by the author (R. L.) in which the name *Coccidium Rivoltae*, Grassi is used for the first time. Railliet (1895, p. 146) uses the name *Coccidium bigeminum* Stiles, 1891, for the small coccidium of the cat, dog and pole cat, and the name *Coccidium* (?) *Rivoltai* Grassi, 1881, for the form of intermediate size seen by Grassi. Neveu-Lemaire (1912) employs the name *Eimeria Rivaltai* Grassi, 1881, for the latter form, while Brumpt (1922), in the latest edition of his *Précis de Parasitologie*, uses the name *Isospora Rivoltai* Grassi, for all these parasites.

Several observers, including Wasielewski (1904), Martin (1909), Guiart (1910) and Hall and Wigdor (1918), place these parasites in the genus *Diplospora*, which, however, is generally recognised as a synonym of *Isospora*.

For convenience of reference, the following list of names

which have been employed for the *Isospora* of cats and dogs is appended :—

Finck (1854). Corpuscules géminés.
Vulpian (1858). Corps oviformes.
Ercolani (1859). ? (Quoted by Rivolta and Perroncito.)
Virchow (1860). Psorospermien.
Leuckart (1860). Psorospermien.
Davaine (1860). Corpuscules géminés.
Leuckart (1863). Psorospermien.
Leuckart (1866). Psorospermien.
Eimer (1870). Psorospermien.
Zürn (1874). Psorospermien.
Rivolta (1874). Cellule oviforme.
Rivolta (1877). Cellule oviforme.
Rivolta (1877). Cellule oviforme.
Davaine (1877). Corpuscules géminés.
Rivolta (1878). *Cytospermium villorum intestinalium canis.*
Leuckart (1879). *Coccidium perforans.*
Grassi (1879). *Coccidium Rivolta.*
Grassi (1882). *Coccidium Rivolta.*
Bütschli (1882) *Coccidium Rivolta* Grassi.
Perroncito (1882).
Coccidium Rivolta.
Cytospermium villorum intestinalium canis.
Braun (1883). *Coccidium perforans.*
Grassi (1883). *Coccidium Rivolta.*
Balbiani (1884). *Coccidium perforans.*
Leuckart (1886). *Coccidium Rivoltae*, Grassi.
Railliet (1886). *Coccidium Rivolta* Grassi.
Pachinger (1887). Sporozoon.
Neumann (1888). *Coccidium perforans.*
Railliet and Lucet (1888). Corps oviformes.
Zürn (1889). *Coccidium oviforme* Leuck.
Blanchard (1889). *Coccidium Rivolta* Grassi, 1881.
Pfeiffer, L. (1890). Coccidien.
Railliet and Lucet (1890). Coccidies.
Stiles (1891). *Coccidium bigeminum.*
Pfeiffer, L. (1891). Coccidien.
Railliet and Lucet (1891). *Coccidium bigeminum* vars. *canis*, *cati*, *putori.*
Stiles (1892). *Coccidium bigeminum* Stiles, 1891.
Neumann (1892).
Coccidium bigeminum.
Coccidium perforans.
Coccidium Rivolta Grassi.
Mosler and Peiper (1894). Coccidien.
Railliet (1895).
Coccidium bigeminum Stiles, 1891.
Coccidium(?) *Rivoltai* Grassi, 1881.
Braun (1895). *Coccidium bigeminum* Stiles 1891.
Moniez (1896). *Coccidium bigeminum* Stiles (1891).
Blanchard (1896). *Coccidium bigeminum* Wardel Stiles, 1891.
Labbé (1896). *Coccidium bigeminum* Stiles.
Wasielewski (1896).
Coccidium bigeminum Stiles.
Coccidium spec. inc. *Rivolta* Grassi.
Labbé (1899). *Coccidium bigeminum* Stiles.
Blanchard (1900). *Coccidium bigeminum* Wardell Stiles, 1891.
Neveu-Lemaire (1901). *Coccidium bigeminum* Wardell Stiles, 1891.
Doflein (1901). *Coccidium bigeminum* Stiles.
Perroncito (1901).
Coccidium bigeminum Wardell Stiles, 1891.
Coccidium Rivolta.
Neveu-Lemaire (1902). *Coccidium bigeminum* Wardell Stiles, 1891.
Neveu-Lemaire (1903). *Coccidium bigeminum* Wardell Stiles, 1891.
Braun (1903). *Coccidium bigeminum* Stiles, 1891.
Minchin (1903). *Coccidium bigeminum* vars. *canis*, *cati*, *putori* Railliet et Lucet.
Wasielewski (1904). *Diplospora bigemina.*
Neumann (1905).
Coccidium bigeminum.
C. Rivoltae (Grassi).
Guiart and Grimbert (1906). *Coccidium bigeminum* Stiles.
Lühe(1906). *Isospora bigemina* (Stiles).
Braun (1906). *Coccidium bigeminum* Stiles, 1891.

Neveu-Lemaire (1906). *Coccidium bigeminum* Wardel Stiles, 1891.
Braun (1908). *Isospora bigemina* (Stiles) 1891.
Neveu-Lemaire (1908). *Coccidium bigeminum* Wardel Stiles, 1891.
Basset (1909). *Diplospora bigemina.*
Guiart and Grimbert (1908). *Coccidium bigeminum* Stiles.
Braun and Lühe (1909). *Isospora bigemina* (Stiles).
Martin (1909). *Diplospora bigemina* Stiles.
Doflein (1909). *Isospora bigemina* (Stiles).
Braun and Lühe (1910). *Isospora bigemina* (Stiles).
Brumpt (1910). *Coccidium bigeminum* Wardel Stiles, 1891.
Guiart (1910). *Diplospora bigemina.*
Doflein (1911). *Isospora bigemina* (Stiles).
Fiebiger (1912). *Isospora bigemina* Stiles.
Neveu-Lemaire (1912). *Eimeria Rivoltai* Grassi, 1881.
Jollos (1913). *Isospora bigemina.*
Brumpt (1913). *Coccidium bigeminum* (Wardel Stiles, 1891).
Swellengrebel (1914). *Isospora bigemina* (Stiles).
Braun and Seifert (1915). *Isospora bigemina* (Stiles) 1891.
Doflein (1916). *Isospora bigemina* (Stiles).
Fantham (1916). *Isospora bigemina*, Stiles, 1891.
Wenyon and O'Connor (1917). Isospora of cats.
Hall and Wigdor (1918). *Diplospora bigemina.*
Dobell (1919).
Isospora bigemina Stiles.
Isospora rivoltae Grassi (1879).
Reichenow (1921). *Isospora bigemina* (Stiles).
Dobell and O'Connor (1921). *Isospora rivoltae* Grassi.
Nöller (1921). *Isospora bigemina.*
Mayer (1922). Coccidien ?
Brumpt (1922). *Isospora Rivoltai* Grassi.
Marotel (1922). *Isospora cati.*

DESCRIPTION OF THE COCCIDIA OF CATS AND DOGS

During the course of certain observations on the faeces of dogs, the results of which have been published by Brown and Stammers (1922), it became evident that dogs were sometimes infected with a species of *Eimeria* in addition to the commonly recognised *Isospora*. The oocysts of the latter parasite agreed, as regards dimensions, with those given by Grassi (1879, 1882, 1883) for the *Isospora* of the cat, by Wasielewski (1904) for the *Isospora* of the dog and small form in the cat, and by Reichenow (1921) for one in the dog, and were constantly smaller than the oocysts of the *Isospora* of the cat which was under observation at the same time, so that it seems highly probable that the common *Isospora* of dogs and cats belong to two distinct species. The oocysts of the *Eimeria* of the dog varied considerably in size, some of them being as large as those of the large *Isospora* found in the cats, while others were smaller even than those of the *Isospora* seen in the dogs. They differed in appearance from the oocysts of the *Isospora* of the same animal and showed a

much greater range in size, but it was only after material had been kept till development of the oocyst had completed itself that it was definitely recognised as an *Eimeria*. It is possible that this *Eimeria* has been seen before and regarded as an *Isospora*, but of this there is no evidence.

In the case of the *Isospora* of the cat the oocysts examined by the writer have been constantly of large size, except in one instance when very much smaller ones were also present. It was found by examination of the small intestine of this cat that the large oocysts were derived from an *Isospora* (*Isospora felis*) which was undergoing development in the epithelium of the intestinal villi, while the very much smaller ones belonged to another *Isospora* (*Isospora bigemina*) which was parasitic only in the deeper tissues of the villi. Furthermore, the oocysts of the latter form completed their development in the tissues, whereas those of the large form in the epithelium were in the usual undeveloped condition. The faeces of this cat had been examined on several occasions in connection with experiments with *Entamoeba histolytica*, but the only oocysts noted in the faeces were the large undeveloped ones of *Isospora felis*. The oocysts of *Isospora bigemina* were not seen in the faeces, and if they had been present to any extent they could not have escaped recognition. They were first detected when a scraping of the wall of the small intestine was made with a view to finding amoebae which had been seen in this situation in another cat with amoebic dysentery. It seems clear that the oocysts of the small form do not escape into the faeces so regularly as do those of the large one which develops in the epithelium. There is no doubt that there were two distinct species of *Isospora* in this cat.

A detailed study of the development of *Isospora felis* and *Isospora bigemina* as they occurred in the tissues of cats was undertaken, but *Isospora rivolta* of the dog was only investigated in the oocyst stages which occurred in the faeces.

ISOSPORA FELIS n. sp.

The only complete account of the development of this common coccidium of the cat is that of Swellengrebel (1914), though Wasielewski (1904) had described the development of the oocyst and had seen other stages in the epithelium. In its main outlines

Swellengrebel's description is correct, but the growth of the microgametocyte was not fully traced. The supposed parthenogenesis of the macrogametocyte is capable of another interpretation, while the account of the changes undergone by the nuclei requires revision. It seems, therefore, desirable to redescribe the life-history as it has been studied in sections of the epithelium of the small intestine of cats.

Schizont. The smallest forms which can be found in the epithelial cells are only 5 microns in length (Plate IX, fig. 1). These are curved and somewhat sickle-shaped bodies which are pointed anteriorly and rounded posteriorly. They lie in vacuoles in the cells, and are attached to the cytoplasm of the cell by the pointed extremity. The nucleus is spherical and has a definite membrane. Within the nucleus is a body which, in staining reactions, does not appear to be rich in chromatin. It is usually applied to the nuclear membrane. In addition the nucleus contains a granular material which is probably chromatic in nature. Whether the large body should be regarded as a karyosome depends on the definition of this term. It does not stain intensely with Mayer's haemalum, has the appearance of plastin material rather than chromatin, and in this respect resembles a nucleolus rather than a karyosome. Growth of the parasite takes place till it has a length of about 10 microns and a diameter at its thickest part of about 5 microns (Pl. IX, figs. 2 and 3). Though plumper than the youngest forms, it still retains its elongate gregariniform character. While still in this condition nuclear division commences (Pl. IX, fig. 4) by division of the karyosome. The daughter karyosomes take up positions at the end of the now elongated nuclear membrane as two polar caps while a definite equatorial plate of small chromosomes is formed (Pl. IX, fig. 5). Two daughter plates are formed and the first nuclear division is completed by division of the membrane (Pl. IX, figs. 6-8). The two daughter nuclei have the same structure as that of the original nucleus. The second nuclear division takes place in a similar manner, as does also the third, though the karyosomes as a rule become smaller with each division (Pl. IX, figs. 9-12). When eight nuclei are present, the parasite has become more definitely ovoid in shape, and eight merozoites are formed by a budding process which leaves a definite residual body. Eight appears to

be the usual number for the merozoites, for the vast majority of schizonts which have been seen are of this type. The size of the merozoites, however, varies considerably even when only eight are present (Pl. IX, figs. 14 and 15). It seems possible that the small forms are destined to develop again into schizonts and the larger ones into gametocytes, but no definite proof of this could be obtained. Occasionally a smaller number of merozoites appeared to be formed (Pl. IX, fig. 16), but in such cases it is possible that the appearance was due to multiple infection of a cell by merozoites after schizogony had occurred, or to the fact that all the merozoites resulting from schizogony had not escaped from the cell. In several instances the occurrence of two merozoites in a single vacuole was undoubtedly due to two merozoites having invaded the same cell simultaneously. On the other hand, a large number of merozoites is sometimes formed, as pointed out by Swellengrebel (Pl. IX, figs. 17 and 18). In several instances as many as sixteen occurred, while a larger number was once seen. These forms, however, occurred rarely in the material examined, and, as stated above, the great majority of schizonts produced only eight merozoites.

It should be pointed out that the schizonts tend to stain very deeply, even with very dilute Mayer's haemalum, which proved to be the most satisfactory stain for these forms, so that unless thin sections are examined there may be considerable difficulty in making out the details of the nuclear divisions.

During growth the schizont is closely applied to the nucleus of the host cell, which becomes definitely altered in character.

Microgametocyte. The microgametocyte possibly commences as one of the larger merozoites (Pl. IX, fig. 19). Like the schizont, it retains, for a considerable period of its growth, its gregariniform character. When it has a length of about 12 microns (Pl. IX, fig. 20) the first nuclear division takes place. This is very similar in character to that of the schizont. The karyosome is present and divides in the same manner by dumb-bell constriction, while there is evidence that chromosomes are also formed (Pl. IX, figs. 20-22). Repeated nuclear divisions of the same type take place while the microgametocyte increases steadily in size. It finally loses its gregariniform character and becomes irregular in shape till it has a length of about 20 microns (Pl. IX, figs. 23-28). The increase in

bulk up to this stage has been relatively enormous. The details of the nuclear divisions are difficult to follow owing to the marked affinity the cytoplasm has for stains. This obscures details to such an extent that it is very difficult to detect the arrangement of the chromatin during the divisions of the nucleus.

After this a change takes place. The cytoplasm ceases to stain intensely, the chromatin material in the nucleus becomes much more definite and the karyosome decreases in size. Nuclear divisions continue, and these are definitely mitotic in character (Pl. X, figs. 1-3). The chromosome number has not been counted with accuracy, but it appears to be somewhere within the limits of 8 and 12. It appears that the nuclear membrane persists throughout nuclear division. The cytoplasm becomes fissured in various ways and loses still more its affinity for stains. Finally, when nuclear division is complete, the microgametocyte contains a large number of nuclei which have definite nuclear membranes within which are irregular masses of chromatin (Pl. X, fig. 4). The karyosome, which had decreased in size during the later divisions, is no longer clearly visible, but it seems probable that it is still present, for in the later divisions of the nucleus it is often possible to detect a small granule at each end of the mitotic figure. These two granules may be united by a fibre, so that the appearance of a minute karyosome dividing by elongation and constriction is produced. The nuclei then shrink, and become compact, deeply staining masses of chromatin (Pl. X, fig. 5).

Formation of microgametes commences by the outgrowth from the nucleus of a short process (Pl. X, fig. 6). The whole nucleus then elongates (Pl. X, fig. 7), and it seems probable that the short process represents the anterior end of the microgamete. The short curved masses then become more elongate, and fine tapering microgametes about 5 microns in length are formed (Pl. XI, fig. 1). The cytoplasm of the microgametocyte either collects into a single large residual body on the surface of which the microgametes lie, or it breaks up into several separate masses. A certain number of deeply staining granules remain in the residual body. The individual microgamete is pointed anteriorly and fine and tapering posteriorly. Sometimes there appeared to be a deeply staining granule near the anterior end of the microgamete. It is possible

that this granule functions as a blepharoblast from which the two flagella which Swellengrebel demonstrated arise. It seems probable that this granule is the karyosome or, as some would term it, the centriole which could be detected during the later divisions of the nuclei of the microgametocyte. When development of the microgametocyte is completed it may have a length of nearly 50 microns and measure over 30 microns in the two other diameters, so that it appears in many sections of a series. Well over two thousand microgametes may be formed by each microgametocyte. Swellengrebel was unable to trace the complete development of the microgametocyte, but it appears from his figures that some of the forms which he regarded as developmental stages of schizonts are really microgametocytes.

Macrogametocyte. It is assumed that the macrogametocyte commences as one of the larger merozoites (Pl. XI, fig. 2). At this early stage it has been impossible to differentiate between the young stages of either the microgametocytes, macrogametocytes or schizonts. The macrogametocyte can, however, be recognised at later stages owing to the fact that it has increased in size without nuclear division. It retains its gregariniform character, and is attached to the surface of the vacuole in the cell by its pointed extremity. The attachment is frequently on the nucleus, which in some cases is drawn into the vacuole (Pl. XI, figs. 3 and 4). On several occasions what appears to be a definite organ of attachment was seen (Pl. XI, fig. 5). Sometimes there is an appearance of a terminal sucker which has drawn into it a small pedicle of the cytoplasm of the cell. Even when the macrogametocyte reaches a large size the gregariniform shape is retained, so that the parasite may become doubled to accommodate itself to the space at its disposal (Pl. XI, figs. 6-8). During the stages of growth represented by Pl. XI, figs. 2-8 the cytoplasm stains deeply, with a tendency towards the accumulation of more intensely staining material round the nucleus in the later stages. The nucleus has increased considerably in size and possesses a large karyosome which has little affinity for stains. A change now takes place in the staining reactions. The deeply staining material round the nucleus increases in amount and there appears in the cytoplasm a number of deeply staining irregular bodies, while the cytoplasm itself becomes filled with vacuoles containing a clear refractile substance

(Pl. XI, fig. 9). The cytoplasm generally has less affinity for stains than it had previously, and it seems as if the substance which caused the cytoplasm to stain deeply in the earlier stages has now become aggregated in the irregular masses. The latter eventually disappear, leaving a clear cytoplasm filled with refractile globules (Pl. XI, figs. 10 and 11; Pl. XII, fig. 1). Finally the oocyst is secreted round the macrogametocyte. It does not become thick or resistant till it leaves the cell, for in fixed tissues the oocysts within the cells are permeable to fixatives and show no signs of the shrinkage and lack of proper fixation which is characteristic of those which are free in the lumen of the intestine.

During the growth of the macrogametocyte it is frequently noted that a granular substance accumulates in the vacuole between the parasite and the wall of the vacuole. This material, which often stains brilliantly with eosin, causes indentations in the macrogametocyte in various places (Pl. XI, fig. 11). Similar accumulations sometimes occur in the case of the microgametocytes (Pl. X, fig. 1).

Swellengrebel described a process of parthenogenesis of the macrogametocyte. Nothing comparable with this has been seen during the present investigations, and, judging from his figures, it seems that the stages he figures, in which definite nuclei are not present, are drawn from sections of macrogametocytes which did not include the nucleus but showed the deeply staining material which occurs around it (Text-fig. 2, p. 259). The large macrogametocytes naturally occur in several sections of a series, and the nucleus may only be found in one of these. In the sections on either side of this one the macrogametocytes will have the appearance of the forms figured by Swellengrebel as illustrating his process of parthenogenesis.

The foregoing description of the development of *Isospora felis* in the intestinal epithelium of the cat is of interest from several points of view. In the first place it is of importance to note that the parasite is limited to the epithelial cells. In no case has it been seen in the sub-epithelial tissues. The infection, moreover, appears to be confined almost entirely to the epithelium near the distal ends of the villi, there being little tendency for it to spread towards their bases.

During the growth of the young forms of the schizont and

gametocyte the parasite retains its gregariniform character to a relatively late stage. In this respect *Isospora felis* differs from many coccidia, which quickly assume the spherical form when growth commences. The fixation of the growing forms to the surfaces of the vacuoles by the pointed end and the development of what appears to be a definite organ of fixation still further increases the resemblance to certain gregarines, such as those of the genus *Lankesteria*.

The development of the microgametocyte merits special attention from the point of view of the behaviour of its nucleus. Schaudinn (1900), in his description of *Eimeria schubergi*, stated that the nucleus of the microgametocyte broke up into a chromidium, the granules of which collected in the form of a number of nuclei on the surface. A similar process was described by him (1902) for *Cyclospora caryolytica*, and again by Schaudinn and Siedlecki (1897) in the case of *Eimeria lacazei*. The majority of observers who have described the development of the microgametocytes of coccidia have followed Schaudinn in supposing that the numerous nuclei are formed from the chromidium into which the single nucleus breaks up. It was shown by Schellack (1912, 1913) and by Schellack and Reichenow (1913, 1915) for a number of coccidia, including the forms with which Schaudinn himself worked, that the latter's statements were incorrect, and that the nuclei of the mature microgametocyte resulted from repeated nuclear divisions from the original nucleus. A similar process had been described by Wasielewski (1904) for *Isospora lacazei* of birds, by Stevenson (1911) in the case of the *Eimeria* of the goat, by Léger and Duboscq (1910) for *Selenococcidium intermedium*, and by Siedlecki (1899) for *Adelea ovata*. It is very doubtful, therefore, if the microgamete nuclei are ever formed from chromidium, as Schaudinn maintained. It seems far more probable that in all coccidia they result from repeated nuclear divisions, as described above for *Isospora felis*.

The structure which has been called the karyosome is present in all the stages of schizogony and in the merozoites. It occurs during the early nuclear division stages of the microgametocyte, but in the later stages is represented by a minute granule. Whether this is to be regarded as a centrosome or centriole is a difficult question to decide. It certainly occupies the position in mitotic division that

a centrosome would occupy, and furthermore, it is probably this granule which occurs at the anterior end of the microgamete, and from it the flagella may originate. The karyosome is constantly present during the growth of the macrogametocyte, though it usually becomes smaller towards its maturity. Whether it disappears before fertilisation takes place has not been definitely determined, but it is certainly present during the nuclear division of the zygote and sporoblast. There was no indication that the karyosome was discharged from the nucleus prior to fertilisation. Though the latter process was not actually observed in stained preparations, in a few cases the nucleus of the fully grown macrogametocyte was elongated. It seems probable that this was an elongation preparatory to fertilisation, and if so it is worthy of note that the karyosome was still present in the nucleus.

Oocyst. As regards the oocysts themselves (Pl. XII, figs. 12-15) the measurements of a large number showed that they vary in length from 39 to 48 microns and in breadth from 26 to 37 microns, the majority measuring about 45 by 33 microns. These figures are practically identical with those given by Swellengrebel. Wasielewski, however, saw smaller forms in the cat, his measurements being 22 to 40 by 19 to 28. It seems possible that cats may be infected with both *Isospora felis* and *Isospora rivolta*, in which case Wasielewski's figures would cover a mixed infection with these two forms. Grassi appears to have been dealing with a pure infection of *Isospora rivolta* in the cat.

The development of the living oocyst of *Isospora felis* has been followed by Wasielewski, Swellengrebel and others, and there is little to add to their descriptions. Owing to the impermeable nature of the oocyst wall, it is difficult to obtain satisfactorily fixed preparations of the nuclei during its development. A certain number of preparations was, however, obtained in the following manner. Small quantities of the material containing oocysts in various stages of development were crushed between a slide and cover-glass in order to rupture the cysts, and films fixed in Schaudinn's fluid and stained with iron haematoxylin were made in the usual manner. There was thus obtained a number of stained preparations of the different stages.

The nucleus of the zygote (Pl. XII, fig. 2) has very much the

same appearance as that of the macrogametocytes in the tissues. A karyosome is still present, though it appears to be smaller. The same type of nucleus occurs in other stages, including those of the sporoblasts (Pl. XII, figs. 3-7), but in these the karyosome has increased relatively in size. A few nuclear divisions were seen, but these were not sufficiently numerous for many details to be made out. The stages which were seen resembled those which occur in the early stages of development of the microgametocyte, except that the karyosomes are smaller. The nuclei in various stages of development of the oocyst are depicted in Pl. XII, figs. 2-10.

The zygote nucleus (Pl. XII, fig. 2) is a spherical body consisting of a definite membrane, within which a number of fine granules and one larger mass—the karyosome—occur. Whether the karyosome is present in the earliest stage of the zygote nucleus could not be determined, as stained preparations of the fertilisation process were not seen. Satisfactory pictures of the first nuclear division were not observed, so that no statement can be made regarding a possible reduction in the number of the chromosomes. The two nuclei of the binucleate stage are shown at Pl. XII, fig. 3. Both nuclei are decolorized, and the small granule at the centre of the karyosome is well seen. The single nuclei of the two sporoblasts have the same structure. The first division in the sporoblast is shown at Pl. XII, fig. 4. The daughter karyosomes occupy the poles of the spindle, while daughter plates of chromosomes are also present. The nuclei of the binucleate stage of the sporoblast are shown at Pl. XII, fig. 5, and here again the nuclei are of the same type. The second nuclear division in the sporoblast shows two spindles with the karyosomes at the poles of the spindle, and definite equatorial plates (Pl. XII, fig. 6). The resulting four nuclei, with somewhat deeply stained karyosomes, are shown at Pl. XII, fig. 7.

Good preparations of sporozoites were fairly numerous. These measured from 10 to 15 microns in length (Pl. XII, figs. 8-11), being smaller after fixation than in the living condition. In some a large vacuole occurs near one end. This is evidently the position of the refractile body often seen in the living sporozoites (Pl. XII, fig. 15). The nucleus was spherical and contained a relatively large karyosome. In specimens from which the stain had been sufficiently extracted (Pl. XII, fig. 8) the karyosome was pale, and at its centre

was a small deeply staining granule. It thus appears that the karyosome is present in all stages of the nuclei during sporogony, though varying considerably in size.

The sporozoites appear to be budded off in pairs from the ends of the sporoblast. Two buds appear at each end, and these grow into elongate finger-like processes into which the nuclei enter. During their growth they turn over the surface of the residual body and lie between it and the wall of the sporocyst.

An important point to note is that the oocyst commences to form as a thin membrane while the macrogametocyte is still within the epithelium, but it does not become a resistant structure till the macrogametocyte has left the cell for the lumen of the intestine. In no case was there any indication that the further development of the contents took place, either in the cells or in the lumen of the intestine. Retraction of the zygote and division of the latter into two sporoblasts, which are the first steps in the development after the oocysts leave the body, were never noted in the case of oocysts within the epithelium or in the lumen of the intestine. It follows, therefore, that whenever observers have described the occurrence of paired bodies in the intestine wall they cannot have been referring to the oocysts of *Isospora felis*.

ISOSPORA BIGEMINA (STILES, 1901)

This coccidium was discovered in one cat which had been employed for experiments with *Entamoeba histolytica*. The cat died during the night, but at the autopsy next morning it was still warm and perfectly fresh and the amoebae active and in healthy condition. The cat had evidently been dead only a short time. It is important to note this fact, for many of the oocysts of *Isospora bigemina* which occurred in the submucosa were fully developed. It seems hardly possible that they could have completed their development in the short time following the death of the cat. This is all the more probable in view of the fact that oocysts of *Isospora felis* which were also present in the intestine were quite unchanged. It can safely be assumed, therefore, that the appearance of *Isospora felis* in the tissues and in the intestine were those which occurred during life. This accords with the descriptions which have been given by Finck, Virchow, Leuckart, Railliet and Lucet, and Stiles.

The sporocysts of this coccidium were first seen in scrapings of the wall of the small intestine after the death of the animal. It was at first thought that they were fully developed sporocysts of *Isospora felis*, but their small size was against this view. Further examination showed that they really occurred in pairs enclosed in an oocyst which was easily ruptured between the slide and cover-glass. The sporocysts had fairly thick, double-contoured walls, and contained four sporozoites and usually a residual body. The oocyst wall enclosing them was of a more delicate nature, and was closely wrapped round the two sporocysts. There was no indication of a micropyle in the oocyst.

Sections of the small intestine showed that the parasite did not occur in the epithelium, but was limited entirely to the sub-epithelial tissues of the villi, especially near their distal ends, some of which were swollen and packed with oocysts in various stages of development. The epithelium contained *Isospora felis*, which on account of its large size contrasted very markedly with the much smaller form in the tissues. Text-figure 2 is from a drawing of a transverse section of a villus, and shows two macrogametocytes, one with a nucleus and the other with the central granular mass to the side of the nucleus, a fully developed microgametocyte with microgametes, and a young micro- or macrogametocyte of *Isospora felis* in the epithelium, and six fully developed oocysts of *Isospora bigemina* in the sub-epithelial tissues.

The earliest stages of *Isospora bigemina* are seen as minute spherical bodies enclosed in vacuoles in the cytoplasm of mononuclear cells (Pl. XIII, fig. 1). Whether these are endothelial cells or not has not been determined. No endothelial cells which were evidently on the walls of blood vessels were found infected. These young forms are barely 2 microns in diameter. They grow into schizonts which are 5 to 6 microns in diameter, and produce about twelve merozoites. Owing to their small size, it is exceedingly difficult to follow the development in the sections (Pl. XIII, figs. 2-4). The microgametocytes have not been definitely identified, though several structures have been seen which are possibly of this nature. One of these has been drawn (Pl. XIII, fig. 5), and it would seem not improbable that the minute curved bodies are microgametes surrounding a residual mass of cytoplasm. The macrogametocyte

develops into an ovoid body 10 to 12 microns in length (Pl. XIII, figs. 6 and 7). It becomes enclosed in an oocyst (Plate XIII, fig. 8), within which it divides into two sporoblasts, which in their turn form sporocysts the walls of which are thicker than that of the oocyst. Within each sporocyst four sporozoites are produced (Pl. XIII, figs. 9-11). In many sporocysts it has been impossible to recognise

FIG. 2. Section of a villus of the cat showing *Isospora felis* in the epithelium and *Isospora bigemina* in the deeper tissues. In the epithelium are seen two macrogametocytes, one cut through the nucleus and one cut to the side of the nucleus: one microgametocyte which has given rise to numerous microgametes and a residual body, and one young form which may be a young macrogametocyte. In the interior of the villus are seen six mature oocysts of *Isospora bigemina*. × 1500.

a residual body, but such a structure is definitely present in some cases. It appears that it breaks up and disintegrates after the sporozoites have been formed.

As regards the fate of the fully formed oocysts, there is no definite information to offer, except that they were not detected during the examination of the faeces made before death. In the sections of the intestine the epithelium was in many cases absent, so that escape

of the sporocysts would be an easy matter if such a change occurred in life. The heavily infected villi were considerably altered in appearance. They were swollen, and an excess of cells was present. It seems probable that such altered villi would break down during life and liberate the oocysts. These would not appear in the faeces regularly, as in the case of *Isospora felis* which develops in the epithelium, but would occur at intervals, whenever a villus broke down sufficiently to discharge its contents, which would include oocysts in various stages of development.

The question of a possible relationship between this parasite and the very much larger *Isospora felis* which develops only in the epithelium has been considered. It might be urged that if *Isospora felis* developed in the sub-epithelial tissues it might take on the character of the smaller form. The latter, however, has only been seen in one animal, while many infected with *Isospora felis* alone have been studied. It seems clear, therefore, that the small form is a distinct species.

The undeveloped oocysts of *Isospora bigemina* have recently been detected in the faeces of a dog by Mr. Leslie Sheather, as noted above.

ISOSPORA RIVOLTA (GRASSI, 1879)

This coccidium has only been studied in the oocyst stage as found in the faeces of three dogs. Mr. Leslie Sheather has also seen the oocysts in the faeces of a dog at the Royal Veterinary College. The oocyst has much the same shape as that of *Isospora felis*, but is smaller. The measurements obtained from the three dogs agree very closely with those given by Grassi (1879, 1882, 1883) for the form in the cat, and Reichenow (1921) for the one in the dog. The dimensions given by Wasielewski (1904) for the oocysts seen by him in the dog are very much the same, as also the smaller series found by him in the cat. Hall and Wigdor (1918) evidently met with this parasite in one dog. The reference made by Nöller (1921) to a large and small form in the cat may refer to *Isospora felis* and *Isospora rivolta*.

Four stages of development of the oocyst are shown at Pl. XIII, figs. 12-15. As seen in English dogs, they vary in length from 20 to 24 microns and in breadth from 15 to 20. Large oocysts

like those of the cat are never seen, though, as pointed out above, when the larger oocysts of *Eimeria canis* were present, it was at first thought that these belonged to *Isospora felis*. The difference in size between the oocysts of *Isospora felis* and *Isospora rivolta* was so constant that there can be little doubt that two species are represented, as Reichenow (1921) has suggested.

EIMERIA CANIS n. sp.

The oocysts of this coccidium were seen in three dogs, as recorded by Brown and Stammers (1922). In two of them the infection was a small one, while in the other it was fairly heavy. The remarkable feature of the oocyst is its great range in size. In this respect it resembles *Eimeria debliecki* of the pig, the oocysts of which were described by Cauchemez (1921). Another feature which is of interest in the case of *Eimeria canis* is that the sporocysts show the same proportional variation in dimensions as do the oocysts. It is evidently incorrect to suppose that in coccidia the sporocysts remain fairly constant in size in spite of variations in the dimensions of the oocysts. The oocyst of *Eimeria canis* varies in length from 18 to 45 microns, and in breadth from 11 to 28 microns. The general shape of the oocyst will be appreciated from the figures (Pl. XIII, figs. 16-19, and Pl. XIV, figs. 1-8). The cyst wall constantly had a peculiar pink colour, and what seemed to be the true oocyst wall was enclosed by a somewhat irregular thick membrane which gradually peeled off during the development outside the body. When this membrane had separated, the colour of the cyst was still the same, though much paler. The course of the development is illustrated in the drawings. It will be noted that a definite micropyle could be detected in some oocysts (Pl. XIII, figs. 16 and 18, and Pl. XIV, fig. 1) and that the enclosed cytoplasm was sometimes attached to it by a strand (Pl. XIII, fig. 16). An inner membrane indicated by radiating lines could also be detected in some of the oocysts (Pl. XIII, figs. 18 and 19, and Pl. XIV, figs. 1 and 2). During the formation of the sporoblasts there was a striking resemblance to *Eimeria stiedae* of the rabbit, as described by Metzner (1903). Pyramidal elevations with clear hyaline apices were formed. The sporocysts had the characters shown in the

drawings (Pl. XIV, figs. 5-8). It will be noted that at the narrower end there is a definite elevation or knob. In many respects the oocysts resemble those of the coccidium of the rabbit. Since the paper by Lucet (1913) appeared, it has been assumed that there are two coccidia in the rabbit, the one, *Eimeria stiedae*, with larger oocysts than the other, *Eimeria perforans*, as first clearly stated by Leuckart (1879). The former, according to Reichenow (1921), who agrees with this view, occurs in both the liver and intestine. In some cases the liver alone is infected, in others only the intestine, while in other animals both are found to harbour the coccidium. The other form, *Eimeria perforans*, is apparently limited to the intestine, though information on this point is not very definite. There seems, however, no reason to suppose that the coccidium of the dog represents two species, though in many respects it corresponds with a mixed infection of two forms in the rabbit. The great variation in size of the oocysts of *Eimeria canis* raises the question as to whether there are actually two coccidia in the rabbit or only one.

It does not seem possible to identify the form in the dog with the common rabbit coccidium, though Bruce (1919) has described a coccidium of the rabbit in America the oocysts of which resemble those of *Eimeria canis* in the presence of the layer of material covering the wall, in its pinkish orange colour and the marked range in size. Bruce was inclined to regard this parasite as a new species, or a variety of the common rabbit coccidium. It certainly resembles *Eimeria canis* more than any other recorded coccidium.

It should be mentioned, however, that Guillebeau (1916) has described a still smaller coccidium, which he says occurs in the liver cells of dogs. He identified it with *Eimeria stiedae*, though the oocysts measured only 12 by 7 microns. As pointed out by Reichenow (1921), the situation of the parasite in the liver cells is a most unusual one for coccidia. The figures given by Guillebeau do not assist in arriving at a conclusion as to the nature of the organism. Chierici (1908), quoted by Martin (1909), recorded a coccidium which he found in the bile of a cat. The oocysts had a thick, double-contoured wall, were oval in shape, and measured 26 to 30 microns in length by 17 to 20 microns in breadth. Development with the formation of four sporocysts, each with two sporozoites,

occurred. It is evidently a coccidium of the genus *Eimeria*, but whether it is identical with *Eimeria canis* cannot be determined.

Virchow (1865, p. 356) records his discovery in the gall-bladder and bile ducts of one dog of numerous egg-shaped psorosperms with thick, double-contoured shells. No further description is given, so that it is not possible to form an opinion as to whether these were oocysts of coccidia or eggs of a trematode. Another reference to similar structures is by Rivolta (1878), who gives the name *Cytospermium hepatis canis familiaris* to certain oval bodies which Perroncito had found in the bile ducts of the dog and which he had called *cellule oviforme del fegato del cane*. Perroncito (1882, p. 98) refers to what are evidently these bodies as '*Citospermio del fegato del cane*.' He gives also the name '*Cellule oviforme del fegato del cane, Perroncito*.' They are described as measuring 48 to 52 microns in length by 21 to 32 microns in breadth. There is a capsule 2 microns in thickness, and at one pole an operculum. The contents divide into two to eight masses. There is little doubt that these bodies are eggs of a trematode. It appears that the first reference was made by Perroncito (1876), but this paper has not been consulted.

ISOSPORA OF MAN

The facts which have been explained above have a direct bearing on the status of the *Isospora* which has been recorded from human beings. It will be necessary to review the history of the discovery of the parasite. The first record of the occurrence of such a coccidium is that of Virchow (1860, p. 527), who mentions a case which was brought to his notice by Kjellberg. He found at post-mortem Psorosperms in the villi, which agreed entirely with those that he (Virchow) had seen in dogs ('*welche ganz mit denen übereinstimmen, die ich beim Hunde gesehen habe*'). The Psorosperms occurred in the interior of the villi, and especially towards their ends ('*in dem Innern und zwar gegen die Spitze der Darmzotten*'). Of the form seen by him in the dog, he says that in the interior of the villi he saw numerous Psorosperms of relatively small size regularly arranged in pairs with a double-contoured membrane ('*Indess habe ich neulich erwähnt* (S. 342), *dass ich in einem Hunde im Innern der Darmzotten sehr haüfig Psorospermien*

antraf; es waren relativ kleine, regelmässig zu zweien aneinandergesetzte mit starker, doppeltcontourirter Membran versehene Körper'). He goes on to state that they must have been like the forms seen by Finck in the cat. From Virchow's statements, the only conclusion justifiable is that he saw in man a small coccidium like *Isospora bigemina* of the cat and dog.

The next reference is that by Eimer (1870), but this is much less satisfactory than that of Virchow. Eimer says that he saw Psorosperms in two men who were examined post-mortem in Berlin. The intestinal canal was described as being filled and the epithelium completely infiltrated with Psorosperms. He says they were like those seen by him in mice and other animals. In both the human cases the epithelium of the greater part of the intestine is described as having been devoured by the Psorosperms, as occurs in infected mice. The contents of the Psorosperms were finely granular. Eimer furthermore states that he observed all stages of the division of the contents, but gives no clear account of the process. From these meagre details it appears impossible to identify the Psorosperms seen by Eimer. Whether they were coccidia at all is far from clear. They evidently did not show the same arrangement in pairs noted by Virchow, for such a striking appearance would hardly have escaped his notice. The only points in favour of the view that they were coccidia are the statements that they occurred in the epithelium, and that they resembled undoubted coccidia of the mouse and other animals. As coccidia belonging to both the genera *Isospora* and *Eimeria* occur in man, it is fruitless to speculate as to which genus the form seen by him belongs.

Rivolta (1873) describes certain corpuscles he found in the faeces of man, but there is no evidence whatever that these were oocysts of coccidia. Similarly, the bodies seen by Grassi (1879), and which he regarded as coccidia, were probably cysts of *Giardia*. Rivolta (1879) proposed the name *Cytospermium hominis* for the psorosperms found in man by Eimer. The name is given explicitly to Eimer's psorosperms, and Rivolta makes no mention of the bodies originally described by him in 1873. Thus Rivolta's name *Cytospermium hominis* was given to certain bodies seen by Eimer which may or may not be coccidia, and even if they were coccidia are quite unidentifiable.

Railliet and Lucet (1890) described the small coccidium of the villi of dogs. They recognise in these the form named *Cytospermium villorum intestinalium canis* by Rivolta (1878). They correctly followed the development with the production of two sporoblasts, each of which gave rise to a sporocyst containing four sporozoites. The oocysts measured 12 to 15 microns by 7 to 9 microns. They state that they had seen coccidia in the faeces of a woman and her child who were suffering from chronic diarrhoea. The coccidia were regularly ovoid, and some of them contained granular protoplasm, including a number of refringent globules. Others contained a large granular mass without globules. The average size was 15 by 10 microns. They recognise, however, that they differed in certain respects from the forms seen in the dog.

In a later paper, Railliet and Lucet (1891) accept the name *Coccidium bigeminum* given by Stiles (1891) to the small coccidium of the dog. As pointed out above, they recognised three varieties of this organism, *Coccidium bigeminum* vars. *canis*, *cati* and *putori* in the dog, cat and pole cat, respectively. They say that a fourth variety probably also exists, namely, *Coccidium bigeminum* var. *hominis*, the form which was seen by Kjellberg and described by Virchow (1860). They make no mention of the bodies described by themselves in 1890. Railliet (1895), however, ascribes to the species *Coccidium bigeminum* the often quoted parasite discovered by Kjellberg. As regards the bodies seen by Railliet and Lucet (1900) in two human cases, Railliet groups them with those described from man by Grassi and Rivolta as doubtful forms about which it is not possible to express an opinion. He states, however, that the size of those recorded by Railliet and Lucet (15 by 10 microns) brings them into relation with *C. bigeminum*. Six pages further on in his book, Railliet again asserts that the parasite discovered by Kjellberg must without doubt be placed in this species (*C. bigeminum*), as it was situated in the interior and towards the tips of the villi, and resembled the form seen by Virchow in the dog. It is thus evident that Railliet and Lucet, in employing the name *Coccidium bigeminum* var. *hominis*, were naming not the form seen by themselves, but Kjellberg's parasite recorded by Virchow (1860).

From what has been said above, it will be apparent that in only one of the records, namely that of Virchow, is it possible to make

an accurate deduction that a coccidium was being dealt with. Rivolta's name *Cytospermium hominis* refers to Eimer's parasite which cannot possibly be identified. If a coccidium at all, it may have been an *Isospora* or an *Eimeria*, but nothing more definite can be asserted. In the case recorded by Virchow, however, we know that he was familiar with the small *Isospora* of the dog. He recognised that the latter occurred in the tissues of the villi and not in the epithelium, and that it occurred in pairs and was like the parasite of the cat described by Finck. Of the human form, he says it occurred in the interior of the villi, especially towards their distal ends, and that it agreed entirely with the one he had seen in the dog. The only possible conclusion which can be drawn legitimately from these precise statements is that Virchow actually meant what he said and was observing in man a small *Isospora* like *Isospora bigemina* of dogs and cats. With the very doubtful exception of the bodies seen by Railliet and Lucet (1890) this small coccidium has not since been discovered. At first sight this may seem surprising, but there appears to be a possible explanation. When Finck made his observations on the cat he was concerned mostly with the changes undergone by the intestinal epithelium during digestion rather than with the faeces. He was actually examining the intestinal wall itself, and not the dejecta of his animals. Similarly, Virchow and Rivolta, who saw the small *Isospora* of dogs, were concerned mostly with the wall of the intestine, and the same appears to be true of Railliet and Lucet, and Stiles. As pointed out above, the presence of *Isospora bigemina* in the cat was only detected by the writer when scrapings were made from the intestinal wall. In these scrapings the thick-walled sporocysts, often arranged in pairs enclosed by a common membrane, were very striking objects, whereas the incompletely formed oocysts of the large *Isospora felis* which were also present were not nearly so easily seen, and might readily have been mistaken for enlarged tissue cells. If examination in this case had been limited to the faeces the small forms would have been missed entirely, and only the oocysts of the large form seen.

Grassi, however, was concerned largely with the examination of the intestinal contents and faeces, with the result that he discovered the oocysts of the intermediate sized *Isospora rivolta* in the cat.

When he examined the intestinal epithelium he noted that they were present in the epithelial cells, but there was no indication of a paired arrangement as in the case of the small *Isospora bigemina* seen by Finck and others. Since Grassi's time, Wasielewski and other observers, who have likewise studied the faeces, have noted in cats and dogs both *Isospora rivolta* and *Isospora felis*, but never the small *Isospora bigemina*. The developmental stages of the larger forms have been seen only in the epithelial cells, and never in the paired condition in the tissues of the villi. It is not improbable that the tissue-invading small form has been frequently missed owing to failure on the part of investigators to examine scrapings from the intestinal wall itself. Virchow discovered the small form in man because he adopted this method, and it is probable that it would have been re-discovered in recent years had this practice been continued and if examinations had not been limited to the faeces alone.

During the extensive examination of faeces of men necessitated by the exigencies of the war, the oocysts of an *Isospora* were discovered on many occasions. They were first seen by Woodcock (1915) and then by the writer (1915), who demonstrated their development and proved that they actually belonged to the genus *Isospora*, as had been suggested by Woodcock. In a recent paper, Connal (1922) has shown that over one hundred and fifty cases of infection with this parasite are on record. The oocysts measure from 25 to 30 microns in length by about 12 to 15 in breadth. They thus correspond in size with those of *Isospora rivolta* of cats and dogs. They differ, however, in shape, so that they cannot be identified with the parasite of dogs and cats. From what has been said above, it is evidently impossible to identify this human *Isospora* with the small form (*Isospora bigemina*) of cats and dogs or with the small form (*Isospora hominis*) seen by Virchow in man. The fact that the oocysts appear in the stool in the undeveloped condition is strongly suggestive of a development in the epithelium like *Isospora rivolta* and *Isospora felis* of cats and dogs.

Dobell (1919), in his careful review of the coccidia of man, based his arguments on the assumption that only one *Isospora* occurred in cats and dogs, and under the name *Isospora bigemina* he included the small, intermediate and large-sized forms of these animals.

Hence in his discussion of the name which should be applied to the *Isospora* of man, with every justification he included under the name *Isospora hominis* the small form described by Virchow and the much larger form discovered during the war. When it is realised that the small form in cats and dogs which develops in the tissues of the villi is distinct from the larger forms which develop in the epithelium, this position as regards the human parasites at once becomes untenable. The small *Isospora* of man described by Virchow was named *Isospora bigemina* var. *hominis* by Railliet and Lucet (1891), a name which becomes *Isospora hominis* Railliet and Lucet, 1891. As we have seen, the name *Cytospermium hominis* of Rivolta was given to unidentifiable structures seen by Eimer (1870). Dobell (1919) recognises this latter fact, but adopts the position that it is better to assume that Eimer was actually dealing with the form described by Virchow, and strongly urges that this view be accepted. But this statement was made on the assumption that the small forms in the dog and cat were identical with the larger ones, an attitude which is maintained by Dobell and O'Connor (1921), who employ the name *Isospora rivoltae*. It seems unwise to make this assumption, as there are absolutely no data to indicate the nature of the structures seen by Eimer. It is more logical to adopt the name *Isospora hominis* Railliet and Lucet, 1901, for the small *Isospora* of man, and to regard Rivolta's name *Cytospermium hominis* as a *nomen nudum*.

As regards the large *Isospora* of man, no special name has been given to it, though Savage and Young (1917) employed the term *Coccidium isospora* for this parasite. As pointed out by Dobell (1919), this is evidently a misprint or *lapsus calami*. The intention of the writers was not to introduce a new name, but to refer to a coccidium of the genus *Isospora* in contradistinction to one of the genus *Eimeria*, as coccidia belonging to both these genera had been recorded from man during the examinations for intestinal protozoa made during the war. If, however, it is claimed that the name was correctly presented, then, *Coccidium* being a synonym of *Eimeria*, Savage and Young's name becomes *Eimeria isospora*, and one would have to conclude that they were recording an *Eimeria* of man. There is actually no evidence in the paper that this was not the case, however improbable such a conclusion may be. Their name is, strictly speaking, a *nomen nudum*.

An appropriate name for the *Isospora* of man which figured so largely during investigations on the intestinal parasites of man conducted during the war would be *Isospora belli*. It may at first sight appear to cause confusion to introduce a new name for a parasite which is now generally known as *Isospora hominis*, but Virchow (1860) so definitely referred to a small *Isospora* of man, which was named *Coccidium bigeminum* var. *hominis* by Railliet and Lucet (1891), that to submerge this form by applying the name to a much larger and evidently distinct species which is perhaps more easily detected, is not only contrary to scientific procedure, but is unfair to its discoverer and misleading to future investigators. It seems highly probable that if the method of examination of the small intestine at post-mortem by scrapings from the wall be adopted as a regular procedure the small *Isospora hominis*, first seen by Kjellberg, will be re-discovered.

CONCLUSIONS

1. There occur in cats and dogs three species of coccidia of the genus *Isospora*, namely, *Isospora felis* n. sp., *Isospora rivolta* (Grassi, 1879) and *Isospora bigemina* (Stiles, 1891). The last named is a small parasite of the deeper tissues of the villi of the small intestine, and development of the oocyst may be completed in the vertebrate host, while the two former are larger and are parasitic in the epithelium covering the villi, the development of the oocysts not taking place till they have left the body.

2. It is possible, as maintained by Railliet and Lucet, that there are different varieties of *Isospora bigemina*, namely, *I. bigemina* vars. *canis*, *cati* and *putori* from the dog, cat and pole cat, respectively, but there is at present insufficient evidence to justify the conclusion that they are distinct.

3. The large parasite of the 'swift fox,' described by Weidman as a possible variety of *Isospora bigemina*, does not belong to this species, but is more nearly related to *Isospora felis*. If it is a new species, its name will be *Isospora canivecolis*.

4. The complete development of *Isospora felis* in the epithelium is described. A characteristic feature of the intracellular stages is the gregariniform character of the parasite. Schizonts produce, as

a rule, eight merozoites, but sometimes a larger number. The nuclei of the microgametes are the result of repeated division of the original single nucleus of the young microgametocyte. The karyosome appears to be present in all stages of growth of the parasite. The oocyst wall is not completely formed till the parasite has left the cell, and no change in its contents occurs till the oocyst has left the body.

5. The complete development, including schizogony and sporogony, of *Isospora bigemina* takes place in large cells in the internal tissues of the villi, and here the oocyst is formed and completes its development. Its wall is comparatively thin, while that of the sporocyst is relatively thick.

6. The development of the oocyst of *Isospora rivolta* was studied, and this takes place only after it has left the body, as in the case of *Isospora felis*.

7. The parasite described from the interior of the villi of man by Virchow is a small *Isospora* like *Isospora bigemina*. It bears the name *Isospora hominis* (Railliet and Lucet, 1891).

8. For the larger form discovered in the faeces of man during the war, and regarded by Dobell as identical with the small form described by Virchow, the name *Isospora belli* n. sp., is proposed.

9. A coccidium of the genus *Eimeria* is described from the faeces of dogs. This form is remarkable in that the oocysts vary considerably in size. The name *Eimeria canis* n. sp., is proposed for this parasite.

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ADDENDUM

Since the foregoing account of the coccidia of cats and dogs was written, a paper has come to hand by Zapfe dealing with the *Isospora* of dogs in Germany. The form studied appears to be the one of intermediate size mentioned by Reichenow (1921), and which has been identified as *Isospora rivolta*. It was assumed above that the development of *Isospora rivolta* would be found to take place in the intestinal epithelium, and this has been demonstrated by Zapfe. The various stages are very similar to those of *Isospora felis*, but they are correspondingly smaller, as was to be expected from the smaller size of the oocyst. During schizogony from eight to twenty-four merozoites are produced. The infection is as a rule limited to the distal ends of the villi, as in the case of *Isospora felis*. Zapfe regards the parasite as *Isospora bigemina*, and discusses the statements that have been made as to the occurrence of oocysts in the interior of the villi. He inclines to the view that the oocysts are not actually in this situation, but only appear to be there on account of irregularities in the epithelium. It is evident he has not encountered the small form which unquestionably develops in the interior of the villi.

Reference is made to a paper by Pospiech (1919), which the writer has not seen. This author examined the faeces of a large number of dogs, and came to the conclusion that there were actually four types of *Isospora* in cats and dogs. Three of these correspond with the three forms described above. A fourth type, which occurs in both cats and dogs, has an oocyst which varies in size between that of *Isospora bigemina* and *Isospora rivolta*. The dimensions are given as 17 to 18 microns by 14 microns. The size of the sporocyst is 11 by 7·5 microns. The writer has not seen this form in England, and can express no opinion as to whether it is a distinct species. Zapfe also mentions a paper by Bornhauser (1912), who described a coccidium of the liver of dogs. Nöller is quoted as having expressed the opinion that the structures described were

probably not parasites at all. Reichenow (1921) has come to the same conclusion.

A paper by Otten (1923) refers to the separation of oocysts from the faeces of dogs by a saline concentration method.

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EXPLANATION OF PLATE IX

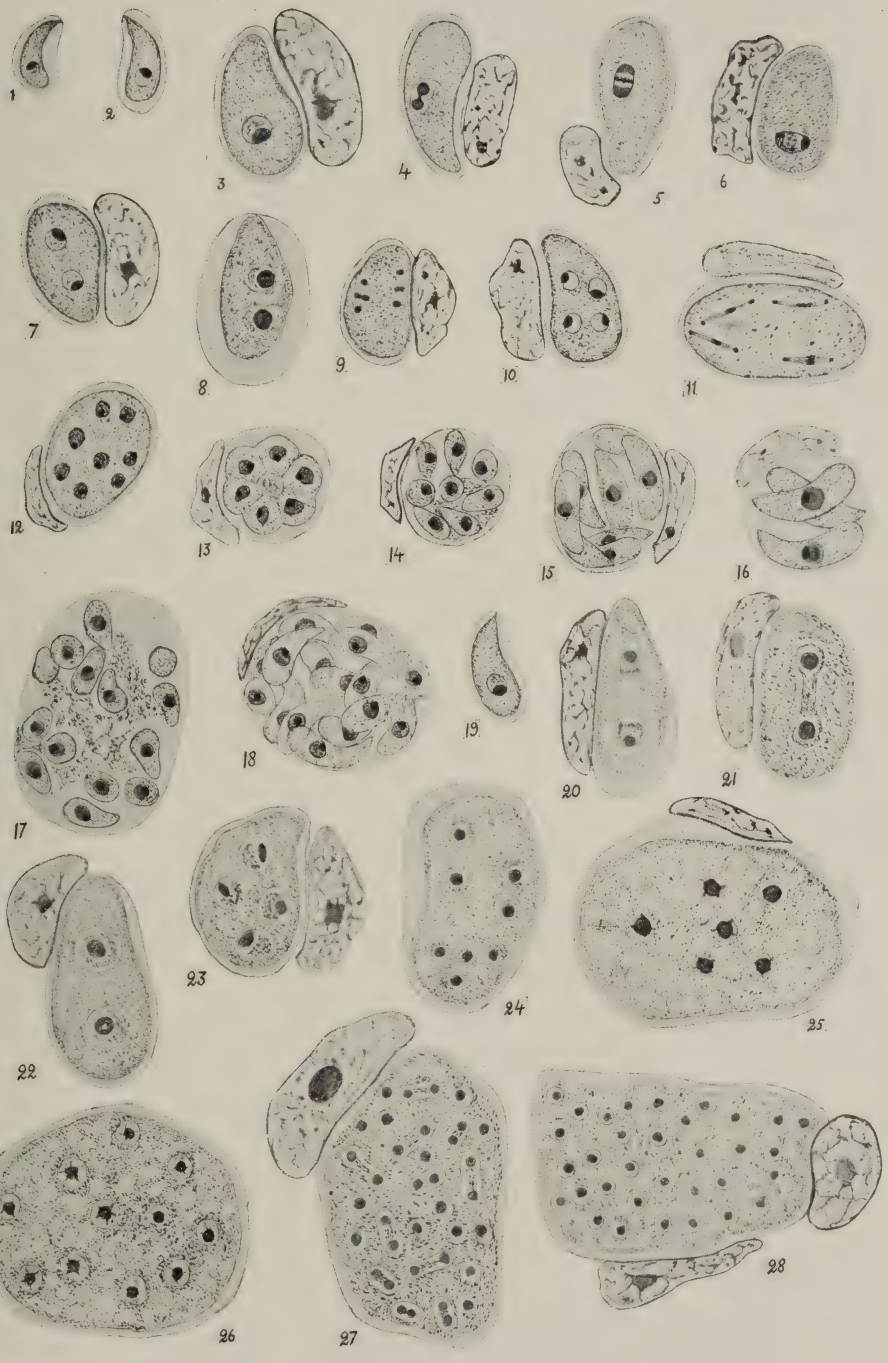
Isospora felis. (× 2000.)

Figs. 1–18. Schizogony.

1. Smallest form in vacuole in epithelial cell showing attachment to surface of vacuole.
2. Slightly larger form with similar attachment.
3. Stage just prior to commencement of nuclear division.
4. Commencing nuclear division. The karyosome in division.
5. Intranuclear spindle showing equatorial plate of chromosomes and daughter karyosomes at ends of spindle.
6. Similar stage showing daughter plates of chromosomes.
7. Stage with two nuclei.
8. Similar stage.
9. Second nuclear division.
10. Stage with four nuclei.
11. Third nuclear division.
12. Stage with eight nuclei. The karyosome is still present though reduced in size.
13. Formation of merozoites from the central cytoplasmic body. Only six of the eight merozoites are shown.
14. Eight merozoites and residual body in vacuole in cell.
15. Eight merozoites of larger size in vacuole.
16. Three merozoites in a vacuole. This is either division into a small number of merozoites or the result of multiple infection.
17. Stage with sixteen merozoites, only fourteen of which appeared in the section.
18. Stage with sixteen larger merozoites.

Figs. 19–28. Growth of microgametocyte.

19. Young microgametocyte ?
20. First nuclear division.
21. Similar form.
22. Stage with two nuclei.
23. Stage with four nuclei.
24. Stage with eight nuclei.
25. One section of stage with sixteen nuclei.
26. One section of stage with about thirty-two nuclei.
27. One section of stage with larger number of nuclei, many of which are dividing. The chromosomes can be detected.
28. One section of stage with still larger number of nuclei.



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EXPLANATION OF PLATE X

Figs. 1–7. Growth of Microgametocyte (*contd.*)

1. One section of stage in which the chromatin has become more distinct, the cytoplasm clearer and the karyosome smaller. Definite mitotic division of the nuclei is taking place.

2. One section of stage in which the chromatin is still more marked.

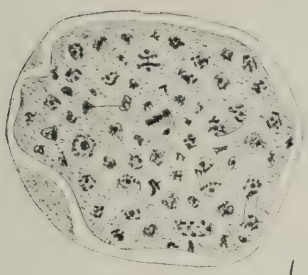
3. One section of stage in which the chromatin is much coarser. Some nuclei are showing what is probably the last nuclear division.

4. One section of stage in which the final nuclear division has taken place. Each nucleus includes several coarse chromatin masses. In some an isolated granule can be detected. This may be the karyosome.

5. One section of stage in which the chromatin granules are becoming aggregated into a single mass.

6. One section of stage in which the chromatin of the nuclei has become completely condensed into a single mass and has formed finger-like outgrowths.

7. One section of stage in which the chromatin of the nuclei has assumed a falciform shape.



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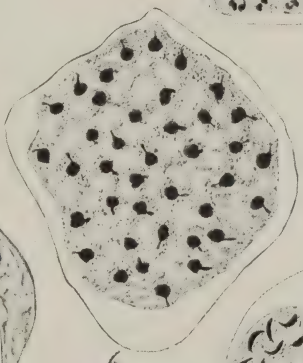

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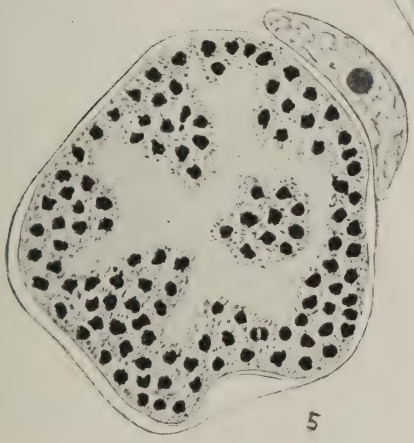

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EXPLANATION OF PLATE XI

Fig. 1. One section of stage in which microgamete formation is completed and several residual bodies are present.

Figs. 2–11. Growth of macrogametocyte.

2. Very young macrogametocyte ?

3. Slightly later stage showing attachment to the surface of the vacuole against the nucleus of the host cell.

4. Later stage showing attachment to the nucleus of the host cell, which has been drawn into the vacuole.

5. Still later stage showing the appearance of a terminal sucker into which a pedicle of the cell cytoplasm has been drawn.

6. Still later stage attached to nucleus.

7. Section of larger form with nucleus of host cell within the vacuole.

8. Section of larger form showing doubled-up condition. The granules of deeply staining material are appearing round the nucleus.

9. Section of later stage. The granules round the nucleus are more marked while deeply staining masses appear in the cytoplasm.

10. Section of larger form. Globules of a refractile substance are appearing in the cytoplasm.

11. Section of a stage in which the globules of refractile substance are more pronounced. The surface is indented in two places by an accumulation of an eosinophile granular material against the wall of the vacuole.

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EXPLANATION OF PLATE XII

Fig. 1. Fully developed stage with clear cytoplasm filled with globules of refractile substance. The oocyst wall is just commencing to form.

Figs. 2–11. Sporogony.

A small area of cytoplasm is figured round each nucleus in Figs. 2-7.

2. Nucleus of the zygote.

3. Two nuclei in zygote after first nuclear division.

4. First nuclear division in a sporoblast.

5. Two nuclei in a sporoblast.

6. Second nuclear division in a sporoblast.

7. Four nuclei in a sporoblast.

8. Sporozoite showing granule at centre of karyosome.

9. Sporozoite with karyosome more deeply stained.

10. Sporozoite showing vacuole in cytoplasm left by solution of refractile body.

11. Stained sporocyst showing four sporozoites and large residual body.

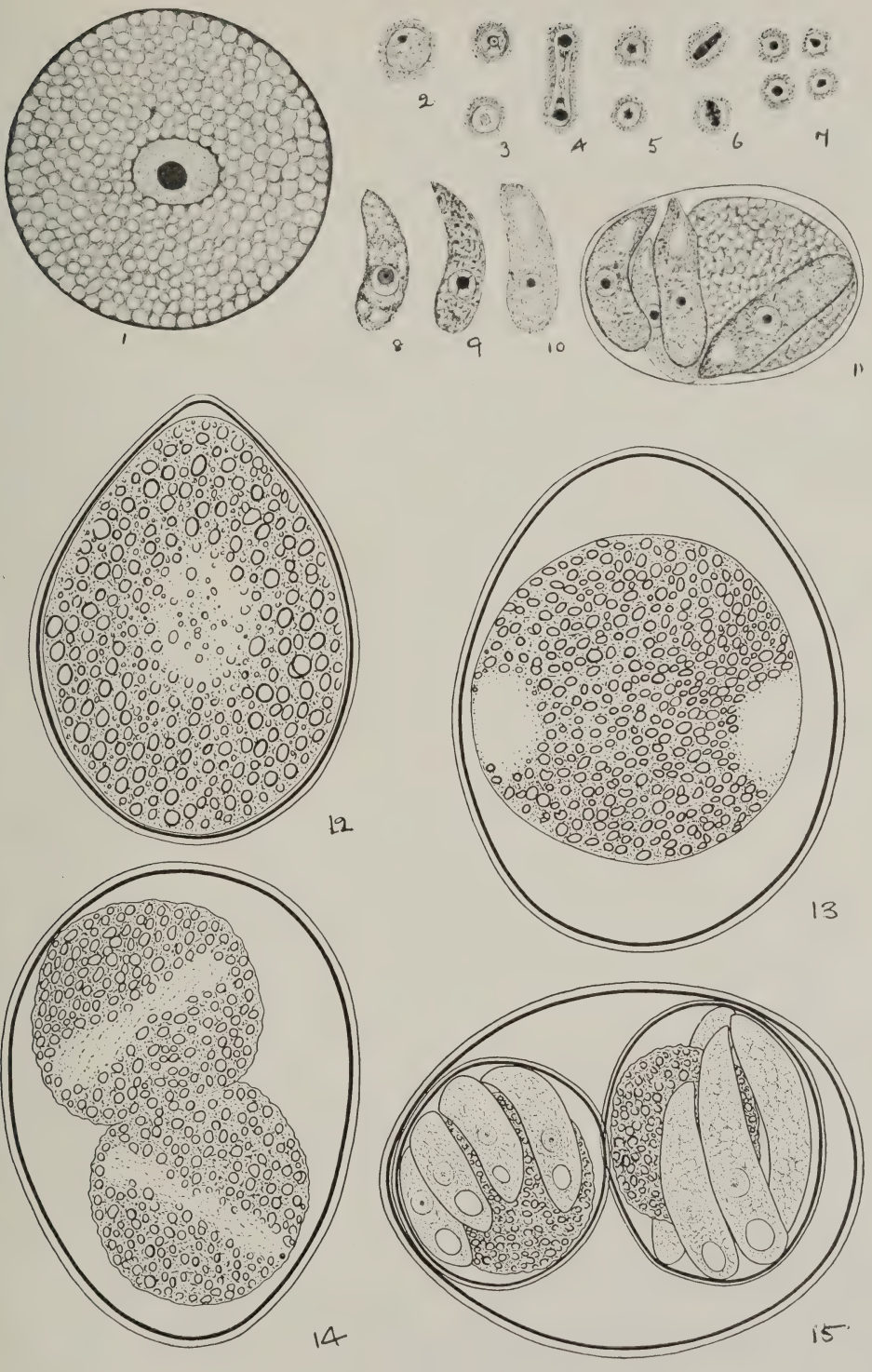
Figs. 12–15. *Isospora felis*—oocysts as seen in living condition. (× 1500).

12. Condition in which oocyst leaves the body.

13. Oocyst in which the zygote has become spherical and the nucleus divided.

14. Two sporoblasts in which first nuclear division is taking place.

15. Mature oocyst showing two sporocysts, each with four sporozoites and a residual body.



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EXPLANATION OF PLATE XIII

Figs. 1–11. *Isospora bigemina.* (× 2000).

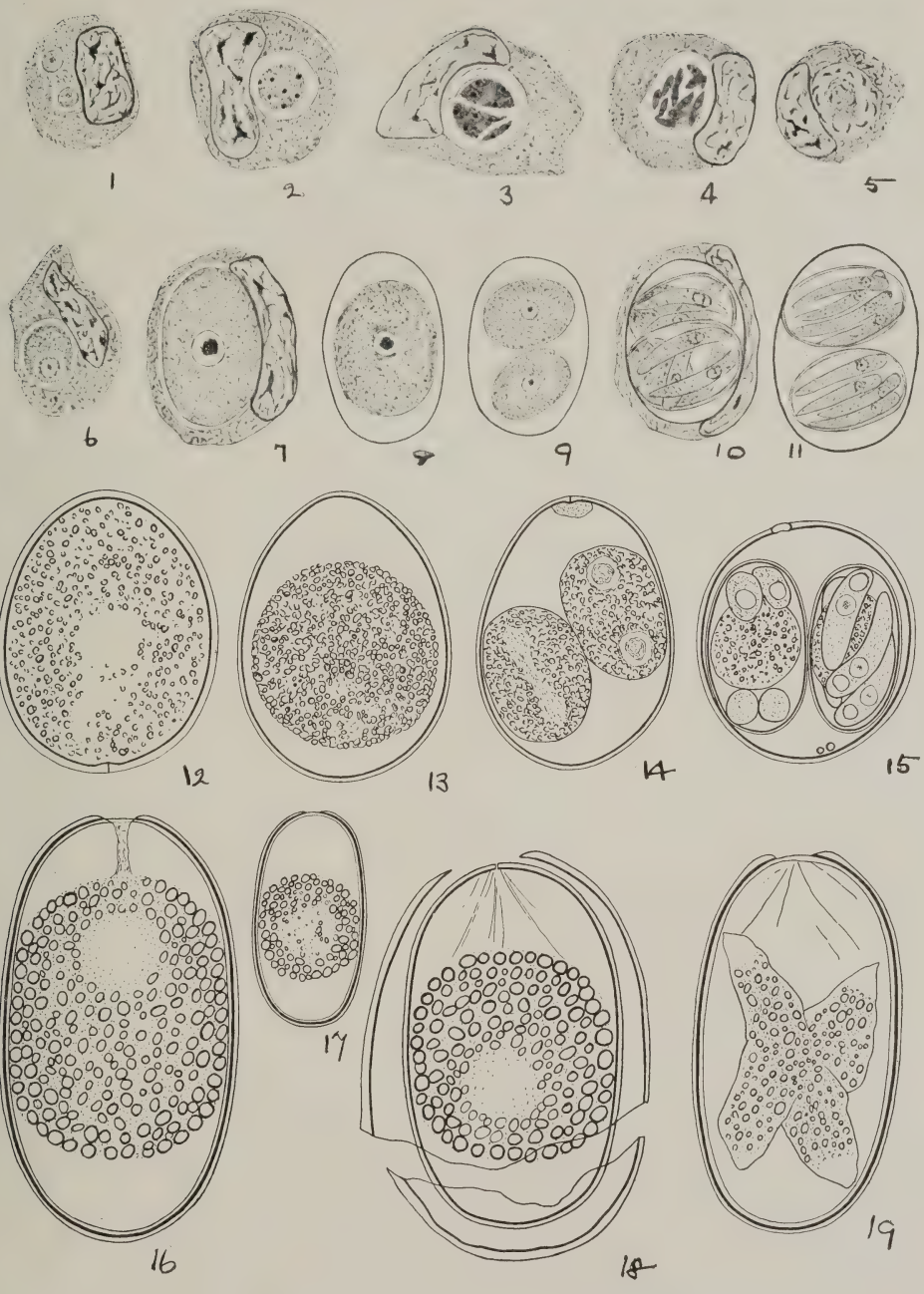
1. Two young schizonts in mononuclear cell.
2. Multinucleated schizont.
3. Commencing segmentation of schizont.
4. One section of stage with about sixteen merozoites.
5. Microgametes and residual body.
6. Partly developed macrogametocyte.
7. Fully grown macrogametocyte.
8. Oocyst with enclosed zygote.
9. Oocyst with two sporoblasts.
10. Fully developed oocyst with two sporocysts, each with four sporozoites and a residual body.
11. Similar stage with no residual body visible in the sporocysts.

Figs. 12–15. *Isospora rivolta*—oocysts as seen in living condition. (× 1500).

12. Condition in which oocyst leaves the body.
13. Oocyst in which zygote has become spherical.
14. Oocyst with two sporoblasts in one of which the nucleus is dividing, while in the other the first nuclear division is complete.
15. Mature oocyst containing fully developed sporocysts.

Figs. 16–19. *Eimeria canis*—oocysts as seen in the living condition. (× 1500).

16. Large oocyst with spherically contracted zygote attached to micropyle by pedicle.
17. Very much smaller oocyst of similar type.
18. Large oocyst with the outer covering breaking away.
19. Oocyst with outer covering intact. The zygote is budding off from sporoblasts as pyramidal bodies.



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EXPLANATION OF PLATE XIV

Figs. 1–8. *Eimeria canis*—oocysts as seen in the living condition (*continued*). (× 1500)

1. Oocyst with outer covering intact. Four sporoblasts and a residual body are present.

2. Similar form with four sporoblasts and no residual body.

3. Oocyst with four elongated sporoblasts and a residual body. The outer covering of the oocyst has disappeared except at two small areas.

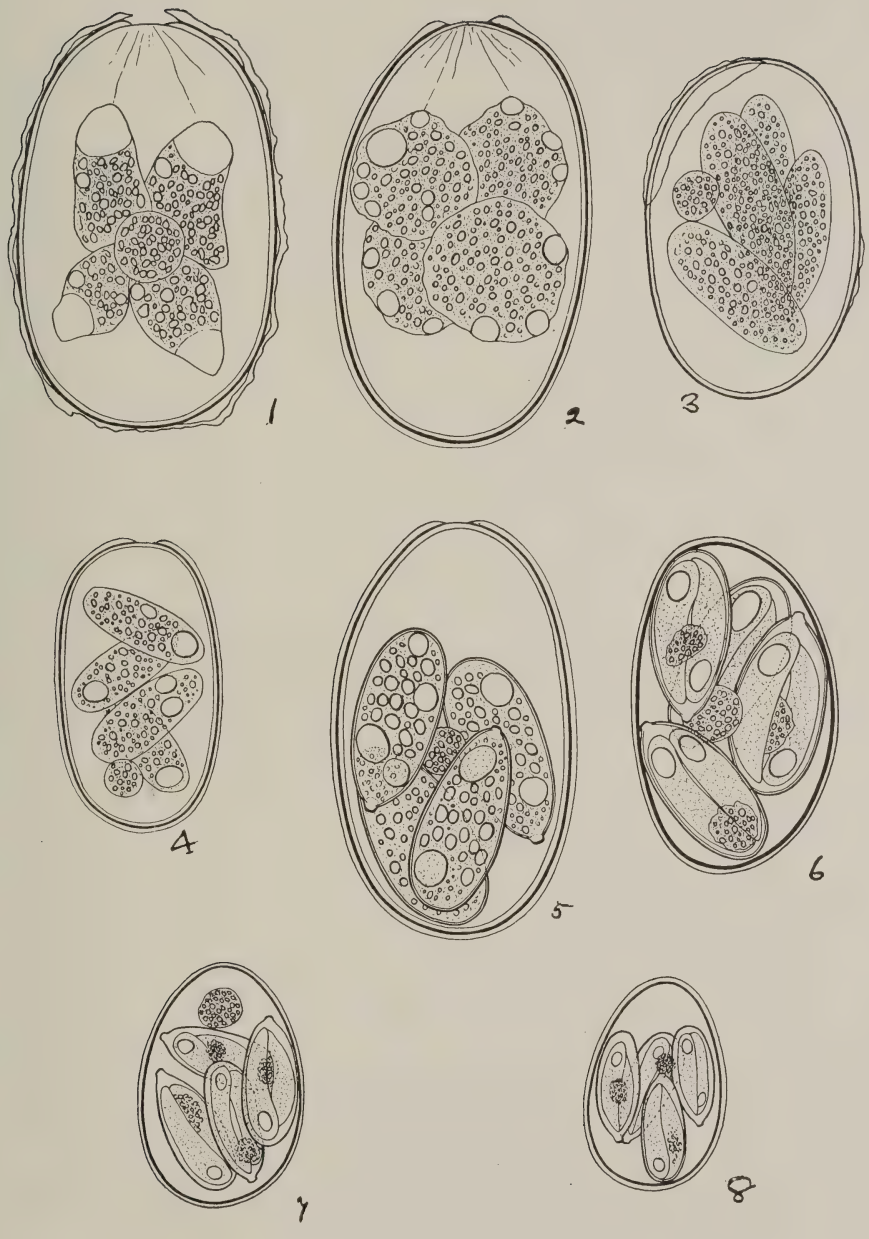
4. Oocyst in similar stage of development with outer covering intact.

5. Oocyst with outer covering intact and four undeveloped sporocysts and a residual body.

6. Completely developed oocyst with residual body and four sporocysts, each of which has a terminal knob and includes two sporozoites and a residual body.

7. Completely developed oocyst of much smaller size.

8. Similar but slightly smaller oocyst.



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A FURTHER NOTE ON THE OCCURRENCE OF ANCYLOSTOMES RESEMBLING *NECATOR AMERICANUS* AMONGST DOMESTIC PIGS IN AMAZONAS

BY

R. M. GORDON

(*Received for publication, April* 25, 1923.)

In a previous note (1922) the author gave a brief description of Necators obtained from domestic pigs in Amazonas and reached the conclusion that, although of smaller size, the worm was indistinguishable from the human parasite *Necator americanus*. About the same time Ackert and Payne (1922) described a hookworm from the gut of the domestic pig in Trinidad. They stated that although this worm resembled *Necator americanus*, it exhibited certain differences which in their opinion were of specific value and accordingly they gave it the name *Necator suillus*. They elaborated their arguments in a later paper (1923). The points on which they base their conclusion that the pig worms differ from the human are the following :—

(1) ' The new species is somewhat smaller.'

(2) ' The buccal capsule much smaller proportionately.'

(3) ' As a rule the dorsal turn in the neck is not so pronounced as in *Necator americanus*.'

(4) ' In *N. suillus* the lateral lancets are broadly wedge-shaped in profile, while those of the large Necator are cusp-shaped. The ventral lancet is slender in side view pointing towards the base of the dorsal tooth in *N. suillus* while this lancet in the larger species is broader and points approximately towards the tip of the dorsal tooth.'

(5) ' The dorsal rays in the new species are shorter, while their

terminal branches are actually longer than these structures in the larger *N. americanus.*'

(6) ' In comparing specimens of these two species, cleared in glycerine, one is struck by the large so-called body cavity in the females of *N. americanus,* as contrasted with a much smaller one in *N. suillus.*'

(7) ' Concerning the males of these two species, the most striking differences are the proportions and shape of the bursa when closed. In *N. americanus* this organ is about as long as wide, and is distinctly funnel-shaped, the distal edges being flared like the bell of a trumpet, while the bursa of *N. suillus* is distinctly longer than wide and is more cup-shaped.'

(8) ' A conspicuous difference between these two species seen under higher magnification is the form of the head papillae. In *N. suillus* each lateral papilla unites with the corresponding dorso-lateral one, enclosing a conspicuous cam-shaped depression, a condition not true of *N. americanus.* Further it may be noted that in *N. americanus* the distal ends of the dorsal, lateral and ventral papillae are more or less beaded or constricted, while in *N. suillus* no such structures occur at the ends of the papillae.'

(9) ' Another rather constant difference is the shape of the externo dorsal ray. In *N. suillus* this ray, which is of nearly equal width throughout its length, makes a sharp lateral turn near its distal end ; while in *N. americanus* the width of this ray is variable and the turn at the tip is less pronounced.'

(10) ' Finally the spicules show constant differences. The average length of the spicules of *N. americanus* is double that of the spicules of *N. suillus.* In the latter species both shafts terminate distally in the membranelle as recurved hooks, while in *N. americanus* only one shaft ends as a recurved hook, the other terminating in a nearly straight line.'

In view of the work of Ackert and Payne the writer has re-examined the pig ancylostomes from Amazonas and compared them with ancylostomes obtained from the human host in Amazonas and Jamaica, with special attention to the points mentioned above.

(1) *Length of the two worms.* Table I shows that, whereas the average size of the pig ancylostome is distinctly smaller, yet its maximum length is equal to the minimum length of the human parasite from Jamaica, and greater than that of the human parasite from Amazonas. The length cannot therefore in itself be used as a distinguishing character.

Table I.

Showing the lengths of Necators obtained from pig and human hosts.

	Males				Females			
	Number measured	Maximum length in millimetres	Minimum length in millimetres	Average length in millimetres	Number measured	Maximum length in millimetres	Minimum length in millimetres	Average length in millimetres
om pig, Amazonas	28	6·5	4·5	5·1	64	8·2	5·5	6·5
om human host, Jamaica ...	28	9·0	6·5	7·8	64	13·0	8·5	10·9
om human host, Amazonas ...	28	8·0	5·0	6·8	64	11·5	7·5	9·1

(2) *Size of buccal capsule.* It appears from Table II that the buccal capsule is proportionately greater in the pig than in the human ancylostome; this is the reverse of Ackert and Payne's findings. Much reliance cannot, however, be placed on small differences in these measurements, as well-marked variations in shape from the normal oval of the

Table II.

Showing the measurements of the buccal capsules in Necators obtained from pig and human hosts.

	Males							Females						
	Number measured	Maximum		Minimum		Average		Number measured	Maximum		Minimum		Average	
		Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.		Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.	Dorso-ventral diam.	Lateral diam.
		μ	μ	μ	μ	μ	μ		μ	μ	μ	μ	μ	μ
pig, Amazonas...	14	66	59	59	51	60	50	31	81	74	66	59	68	57
human host, aica	15	74	57	47	44	59	50	15	85	68	57	54	70	61
human host, azonas	15	64	61	51	44	61	46	15	85	68	64	47	74	60

mouth capsule were frequently encountered; another difficulty is that any variations in the angle from which the head is viewed will give rise to different results in the measurement of the mouth capsule.

(3) *Dorsal curvature of anterior part of body.* No such constant differences as those described by Ackert and Payne were observed in the anterior curvature.

(4) *Ventral lancets, lateral lancets and dorsal tooth.* An examination was made of a large number of worms from both pig and man, but no constant differences in the lancets or dorsal tooth were found; the dorsal tooth and the ventral and lateral lancets of both worms showed great variation in size, shape, and angle of projection, as is illustrated in fig. 1,

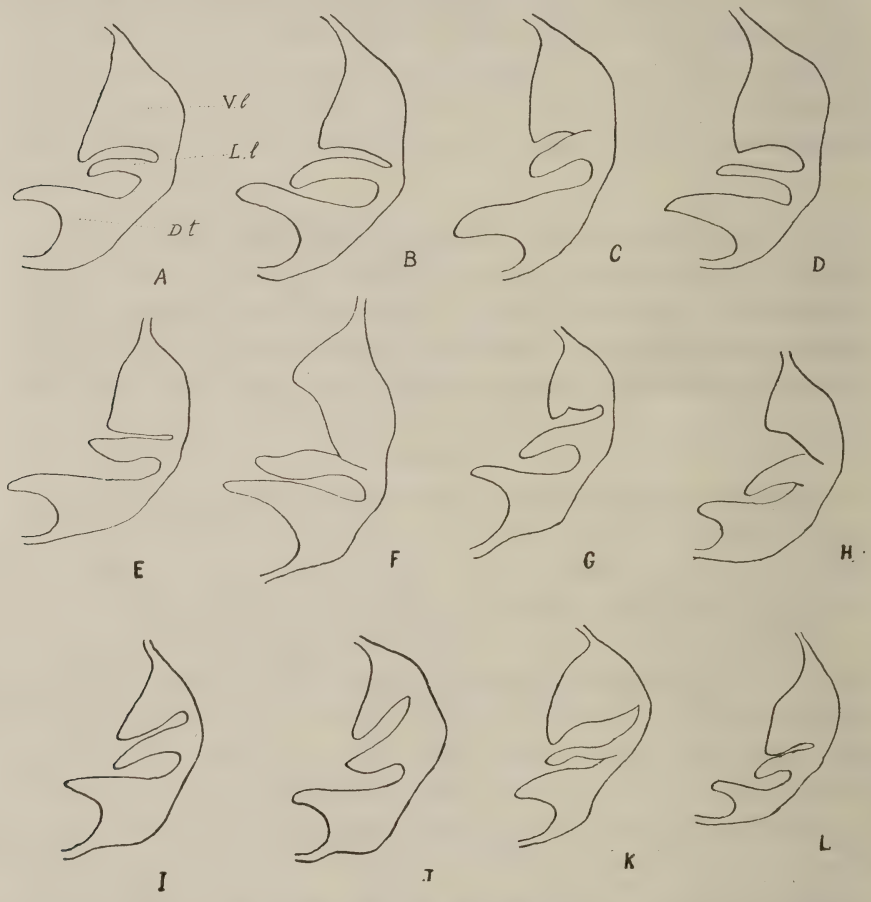

FIG. 1. The three projections in each drawing from above downwards are *V.l.*=Ventral lancet; *L.l.*=Lateral lancet; *D.t.*=Dorsal tooth. *A*, *B*, *C*, *D*, *E* and *F*=*Necator americanus* from human host, Amazonas; *G*, *H*, *I*, *J*, *K* and *L*=Necators from domestic pig, Amazonas.

which represents camera lucida outlines drawn from unselected material. With regard to the angle of projection of the ventral lancets it is of interest to note that Ackert and Payne in their last paper depict these lancets as projecting at almost precisely the same angle in both *N. americanus* and *N. suillus*.

(5) *Length of the dorsal rays.* The results of measuring the dorsal rays in eighteen worms from pigs and in twenty-four from human material are shown in Tables III and IV.

Table III.

Showing measurements of dorsal rays and their branches in Necators obtained from pig and human hosts.

	Length in microns of dorsal ray			Length in microns of inner branch of dorsal ray			Length in microns of outer branch of dorsal ray		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
m pig, Amazonas ...	95·0	65·0	80·0	34·0	18·0	26·4	16·0	10·4	13·9
m human host, maica	130·0	97·0	111·5	36·4	26·0	29·9	19·0	11·7	14·3
m human host, mazonas	120·0	78·5	110·5	32·5	19·5	25·9	15·0	7·8	11·8

Table IV.

Showing ratio of length of dorsal ray and its branches to total length of the worm in Necators obtained from pig and human hosts.

	Ratio of length of dorsal ray to total length of worm			Ratio of length of inner branch of dorsal ray to total length of worm			Ratio of length of outer branch of dorsal ray to total length of worm		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
n pig, Amazonas ...	1 : 57	1 : 92	1 : 71	1 : 183	1 : 333	1 : 215	1 : 343	1 : 528	1 : 402
n human host, maica	1 : 59	1 : 80	1 : 69	1 : 206	1 : 307	1 : 257	1 : 394	1 : 726	1 : 538
n human host, nazonas	1 : 51	1 : 89	1 : 65	1 : 224	1 : 357	1 : 277	1 : 414	1 : 1023	1 : 610

From Table IV it follows that the average of the ratios of the lengths of the branches of the dorsal ray to that of the worm is slightly greater in the case of the pig Necators than in the human, but the maximum and minimum values overlap to such an extent as to make this point of no specific value. From Table III it can be seen that Ackert and Payne's statement that the actual lengths of the branches of the dorsal ray are greater in *N. suillus*, does not hold good for the Amazonas material.

(6) *Size of the body cavity.* Eight worms from the pig contrasted

with eight *N. americanus* from the human host in Brazil, showed no proportionate difference in the size of the body cavity.

(7) *The shape and proportions of the bursae in the two worms.* The dimensions of the closed bursa from its ventral aspect, of six Necators from the pig were 0·26 mm. long by 0·24 mm. broad, while those of six Necators from the human host in Brazil were 0·38 mm. long by 0.41 mm. broad. It is difficult to say when a bursa is completely closed and various stages between cup and funnel shape were observed both amongst Necators from the human and from pig hosts, but these variations in shape appear to depend entirely on the degree of approximation of the two halves of the bursa.

(8) *The head papillae.* The union of the lateral and dorso-lateral papillae as described by Ackert and Payne was clearly visible in the majority of the pig specimens, in others, however, no such union could be traced ; it was, moreover, also present in many of the Necators of man, though possibly not as often as in those of the pig. The beading on the papillae was also found to be a variable factor and was seen at times in both worms.

(10) *Length and shape of the spicules.* The results of measuring the spicules of twelve worms from pigs in Amazonas and of those obtained from the human host in the same locality are recorded in Table V.

Table V.

Measurements of the spicules of Necators obtained from pig and human hosts in Amazonas.

	Length of worm in millimetres			Length of spicules in millimetres			Ratio of length of spicules to total length of worm		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Aver
From pig, Amazonas ...	6·0	4·5	5·3	0·65	0·38	0·47	1 : 8·4	1 : 13·9	1 : 11
From human host, Amazonas	8·7	6·5	7·3	0·98	0·82	0·91	1 : 7·1	1 : 10·6	1 : 8·

It appears from Table V that the average length of the spicules in the worms from man are nearly double the length of those in the worms from the pig. The average of the ratios of the length of the spicules to that of the body are, however, respectively 1 : 8 and 1 : 11, and it must moreover be noted that the maximum ratio in the case of the pig (1 : 8·4) is greater than the minimum ratio in the case of man (1 : 10·6),

and consequently this can hardly be regarded as a reliable point of distinction. Ackert and Payne's statement that both spicules in the pig Necator terminate in the membranelle as recurved hooks in contrast to *N. americanus* in which only one spicule is hooked, was not found to be constantly true of the worms from Amazonas, one hooked and one nearly straight spicule being very common amongst the pig Necators,

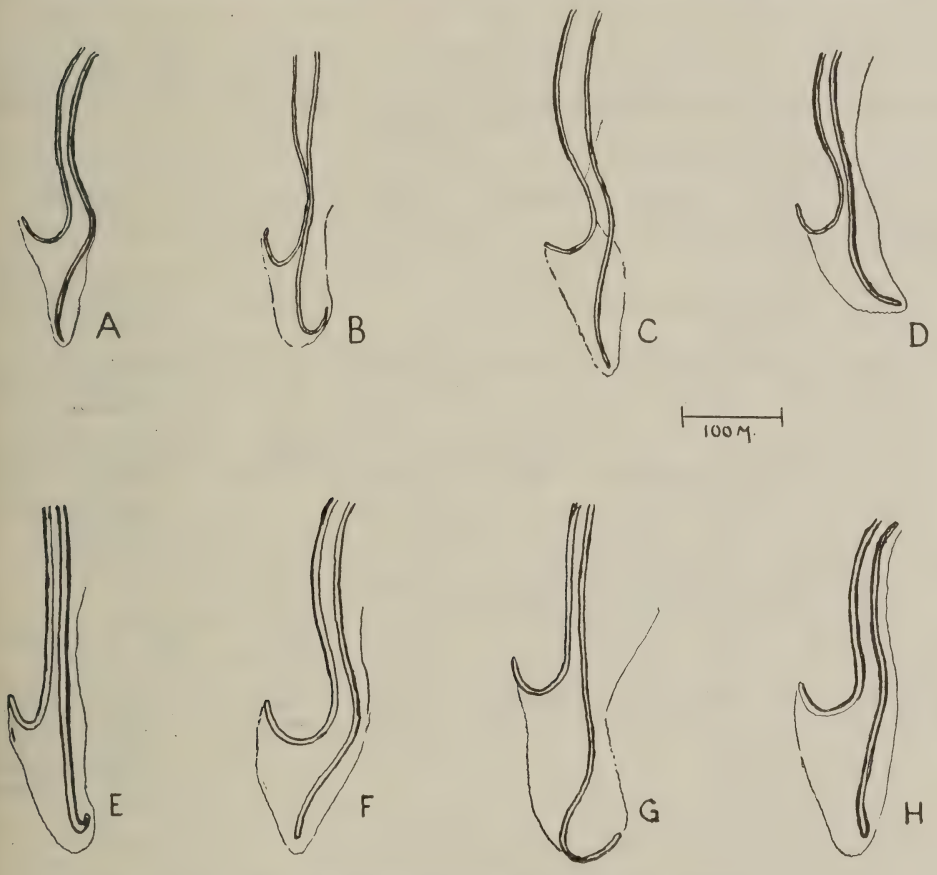

Fig. 2. *A*, *B*, *C* and *D* = Spicules of Necators from domestic pig, Amazonas.
E, *F*, *G* and *H* = Spicules of *Necator americanus* from human host, Amazonas.

while in the case of the Necators from man both spicules frequently showed well-marked hooks. These points are illustrated in fig. 2.

Size of ova. Ackert and Payne give the average size of the ova of the pig Necators from Trinidad as 63μ by 37μ, the ova of the pig Necators from Amazonas were found to measure on an average 64μ by 39μ.

ATTEMPTS TO INFECT PIGS WITH *NECATOR AMERICANUS*

Ackert and Payne (1923) performed several experiments to ascertain whether *N. americanus* from the human host can mature in pigs; they also carried out experiments in pigs with cultures of *N. suillus*. For the sake of brevity, the results of Ackert and Payne's experiments are condensed in Table VI.

TABLE VI.

Summarising the results of Ackert and Payne's attempts to infect domestic pigs with larvae of *N. americanus* obtained from human sources.

No. of pig	No. of larvae	Date given	Stage of larvae	How given	Date killed	Result of autopsy	Remarks
1	2841 2700 3000	16.6.21 20.6.21 27.6.21	'Infective'	Not stated	10.8.21	3 adult *N. suillus*	Faeces free from Nematode ova or larvae at time of experiment
2	2841	16.6.21	'Infective'	Not stated	10.8.21	8 adult *N. suillus*	Faeces free from Nematode ova or larvae at time of experiment
3	1850 12000 4000	27.9.21 29.9.21 4.10.21	'Infective'	By mouth on bread	3.12.21	7 adult *N. suillus*	In spite of oil of Chenopodium treatment, pigs 3, 4, and 5 all had Ancylostome ova at time of experiment
4	7500 9900	29.9.21 1.10.21	Not stated Sheathed	Placed on shaven skin	3.12.21	6 mature *N. suillus*	
5	Nil	—	—	—	3.12.21	3 adult *N. suillus*	Used as a control for pigs 3 and 4
6	5000		'Infective'	Not stated	73 days after infection	No hookworms found	Owing to the difficulty of obtaining pigs free from ancylostomes in Trinidad, this experiment was conducted in Manhattan, Kansas

Ackert and Payne claim to have shown from these experiments that it is not possible to infect pigs with *Necator americanus*, either by the mouth or by the skin. This claim appears to the writer to rest entirely on their ability to distinguish between *Necator americanus* and *Necator suillus*, for in every experiment, except in the case of Pig 6, where the method of administering the larvae is not stated, *N. suillus* was found at the autopsy. It is true that the number of worms found is small (3 to 8),

but on consulting Table VII, it will be seen that equally large doses (6,900 and 23,000) of infective larvae of *N. suillus* resulted in an almost equally small production (10 to 32) of adult worms. It appears certain

Table VII.

Results of Ackert and Payne's experiments at infection of domestic pigs with larvae of *N. suillus* obtained from naturally infected pigs.

No. of pig	No. of larvae	Date given	Stage of larvae	How given	Date killed	Result of autopsy	Remarks
1	2500 4400	29.9.21 1.10.21	'Infective'	By mouth	Died 5.11.21	32 *N. suillus*	Pigs 1 and 2 both showed ancylostome ova in their faeces previous to the experiment for which they received anti helminthic treatment the results of which are not recorded
2	11000 12000	9.10.21 11.10.21	'Infective'	Placed on shaven skin	3.12.21	10 adult *N. suillus*	

therefore that it is extremely difficult to infect the domestic pig with Necators whether the infective larvae used are obtained from other pigs or from the human host.

The present writer while in Amazonas undertook no experiments to infect pigs, owing to the difficulty of obtaining pigs free from Necators and to his inability to distinguish between the pig and the human Necators. One experiment has since been carried out in Liverpool. A pig six weeks old was obtained and kept under observation for seven days; during this period its faeces were examined daily by the saturated salt method (Willis, 1921), but with negative results. On 15.12.22 approximately 400 sheathed larvae obtained from a culture of human faeces were mixed with 2 c.c. of Normal saline and injected subcutaneously in the back of the pig, a similar dose was again given on 29.12.22. The patient from whom these cultures were made was later treated with Carbon tetrachloride and all the 96 worms obtained proved to be *N. americanus*, it therefore appears reasonably certain that the larvae administered to the pig were those of *Necator americanus*. *Trichuris* ova made their appearance in the faeces shortly after the first inoculation and persisted throughout the experiment; no other ova were seen till 19.1.23, when ancylostome-like ova first appeared in the faeces. These ova which were very regular in contour and size, measured 68μ by 37μ, and were quite indistinguishable from those

of the human ancylostomes ; they were always very scanty in numbers, never more than three being found in a single cover slip preparation made from about two grammes of faeces treated by the saturated salt method. Ancylostome ova were not always found at these examinations, the faeces being sometimes negative for two, or even three days. The last occasion on which ova were found present was 5.2.23, examinations on the subsequent four days being negative. The pig was killed 9.2.23, and in spite of a very careful search of the oesophagus, stomach, intestines, trachea, bronchi, etc., the only helminths found were numerous *Trichuris* in the caecum. During the last week of the experiment the whole bulk of the faeces was daily examined for any Ancylostomes that might be passed per rectum, this search proved negative, but it is extremely difficult to detect an odd Ancylostome in a large mass of faeces and it is therefore uncertain whether the pig got rid of the worms responsible for the ancylostome-like ova during the four days prior to the autopsy, or whether they were missed at the post-mortem ; as the search of the organs was undertaken with great care the former conjecture appears the more likely.

CONCLUSION

No constant differences were found between the Necators of Amazonas pigs and those of man from Amazonas and Jamaica such as would justify the formation of a new species for the pig worm.

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A CASE OF ACUTE ASCENDING PARALYSIS IN A CHIMPANZEE

BY

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AND

E. J. CLARK

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(*Received for publication* 15 *November*, 1922)

A male chimpanzee (*Anthropopithecus troglodytes*), judged to be about four years of age, was captured by natives on the 3rd of September, 1922, near Blama, Sierra Leone.

The animal appeared healthy and showed no evidence of injury to the spine. On the evening of the 5th of September the animal was chloroformed in order to be caged, and was then sent down to Freetown. Very little chloroform was used and the animal quickly recovered from the anaesthetic.

On arrival in Freetown, on the 7th of September, two abrasions in the loins, due to the chafing of a rope, were observed; these quickly improved on the application of iodine.

On the 15th of September it was noticed that the animal refused to leave its cage and did not take food; on removal from the cage it was found that the lower limbs were completely paralysed and were colder than the rest of the body. The animal eagerly drank large quantities of milk and water, but refused solid food.

On the evening of the 17th of September the trunk and the upper limbs were completely paralysed; the face was cyanosed and the animal suffered from dyspnoea. Milk feeds were vomited. The muscles of the neck were not affected and the animal could move its head freely from side to side.

On the 18th of September 0·09 gms. of Novarsenobillon were administered intramuscularly into the thigh; there was no loss of

sensation and the animal moved its head vigorously from side to side and attempted to seize the hand of an assistant with its teeth. The animal's condition appeared to improve rapidly after the administration of Novarsenobillon; the cyanosis disappeared and the respiration improved, but it was still unable to swallow solid food and lived entirely on milk.

On the 19th of September the animal passed a solid motion, the first since the 14th of September. As the condition appeared improved after the injection of Novarsenobillon, a second dose of 0·09 gms. was administered on the 20th of September.

No change occurred until the 23rd of September, when general fibrillary twitchings affecting all the muscles of the body were noticed; these twitchings were controlled by an injection of one-eighth grain of morphia. They recurred on the 24th of September, on which day the animal died.

A post-mortem was performed almost immediately after death.

The liver was pale yellowish in colour, and on section showed marked fatty degeneration.

Central nervous system. The cerebro-spinal fluid was slightly turbid and contained a few polynuclear leucocytes. The surface of the brain and cord were congested. Pieces of the cord from the mid-dorsal and upper cervical regions, and pieces of the medulla and cerebral cortex from the motor region (upper and lower limb centres), were fixed in alcohol, embedded in paraffin and sectioned; others were sectioned without embedding. Sections were stained in toluidine and thionin blue and differentiated with alcohol. Eosin and methylene blue, Giemsa, Leishman and Ehrlich's haematoxylin were also used.

Microscopically, the following changes were noticed in the central nervous system:—

Many of the cells in the cord, medulla and cortex were normal and showed Nissl's granules. In the anterior and posterior horns, and in Clarke's column, a number of cells showed faintly staining protoplasm and absence of Nissl's granules, and the nucleus tended to be eccentric in some cells. Vacuolisation of the cell protoplasm was observed in a number of cells; the vacuoles varied in size from 2μ to 6μ, and from one to six were found in each cell. Single vacuoles were found in cells which did not show marked

degeneration, but were noted in large numbers only in cells where degeneration was advanced. The vessels were congested and small haemorrhages were found. Similar changes were noted in the medulla, where vacuolisation of degenerated nerve cells was more marked than in the spinal cord.

Sections of the motor cortex from the upper and lower limb centres showed engorgement of the capillaries. No haemorrhages were found and the gross cellular changes found in the medulla and cord were not seen.

Sections of peripheral nerves showed no pathological changes.

Cultures of the heart's blood were negative.

On the 18th of September 0·2 c.c. of the animal's blood were injected intraperitoneally into a *Cercopithecus campbelli* with negative result.

On the 24th of September, during the post-mortem, 3·5 c.c. of cerebro-spinal fluid were injected intraperitoneally into a *Cercopithecus campbelli*. No paralysis followed. The animal died on the 27th of October, 1922. Post-mortem examination revealed an abscess involving the whole of the upper lobe of the right lung. Smears showed the presence of a Gram-negative capsulated pneumobacillus and a Gram-negative coccus, which was isolated in pure culture.

Before the chimpanzee's illness it had shared a cage with three younger chimpanzees which remained healthy. This, in conjunction with the fact that injection of blood and cerebro-spinal fluid into *Cercopithecus campbelli* produced no paralysis, indicates that the condition was not one of acute anterior polyomyelitis.

The lapse of time between the administration of chloroform and the appearance of symptoms also indicates that delayed chloroform poisoning was not responsible for the condition. Professor Blacklock suggests that the arsenic administered may have contributed to the condition of the liver.

The case presents interest in its close resemblance to the course of acute ascending paralysis as described in human beings.

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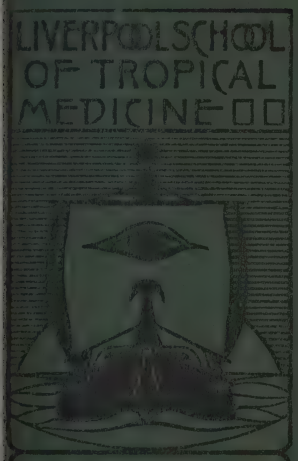

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Annals of Tropical Medicine and Parasitology

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ANNALS

OF

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Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

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STUDIES IN THE TREATMENT OF MALARIA—XXXII

SUMMARY OF STUDIES I—XXXI

BY

J. W. W. STEPHENS

(*Received for publication* 25 *June*, 1923)

The results recorded in the present paper constitute a summary of work on the treatment of malaria carried out at the Liverpool School of Tropical Medicine in the years 1917–1919.

Before considering the various treatments employed, it will be necessary to define certain terms and it will be convenient also to consider certain facts which emerged as the work progressed.

Part I

MALARIA

Only those cases were treated in which parasites were present in the blood at the commencement of treatment. The patient's temperature may, or may not, have been above normal.

RELAPSE

A *parasitic* relapse, febrile or afebrile, i.e., parasites have reappeared in the blood after a negative period induced by treatment.

FEBRILE ATTACKS

A rise of temperature above 100°F., unaccompanied by parasites in the blood within 2–3 days, of which the nature is unknown.

OBSERVATION PERIOD

If we desire to know whether a treatment has cured a patient, i.e., eliminated parasites from his system, it is obvious that the patient must be kept under observation after the treatment. The longer the patient

is kept under observation—by this we mean, not solely clinical observation, but primarily (daily) microscopic examinations of the blood for parasites, the more reasonable will it be to conclude, if the examinations are negative—that he is cured.

The period of observation employed by us was one of 60 days, implying, as we have just said, daily blood examinations. It should be unnecessary to add that *no treatment* was given during the observation period.

FALLACIOUS FIGURES

It is necessary to point out some sources of fallacy in regard to the results of treatment, many examples of which can be found in the literature.

1. *Absence of a microscopic diagnosis of parasites in patients before commencing treatment.* Such cases may be malaria or they may not.
2. *Administration of quinine during the so-called 'observation period'.* The figures relating to relapses are obviously worthless.
3. *Comparison of treatments with different observation periods.*

If the value of two treatments are to be compared, the cases under each treatment must be observed for the *same length of time*, after the cessation of treatment, otherwise the figures for relapses are not comparable, and it is impossible to say which is the better treatment, as in the following example.

Table I.

Treatment	Number of cases treated	Number of cases which relapsed	Number of cases not relapsing but lost sight of before the expiration of 60 days	Number of cases not relapsing in an observation period of 60 days	Relapses	
					Actually observed	Possible maximum
I	100	10	80	10	10%	90%
II	100	30	30	40	30%	60%
III	100	50	0	50	50%	—

4. *Composite figures obtained by summarising the results of various treatments.*

The following is an example :—

Suppose two treatments employed, A and B, and that in the A treatment the relapses were 100 per cent. and that in the B treatment they were 0 ; and further, suppose that 750 cases were under treatment A, 250 under treatment B, then we get the following result :—

	Cases treated	Relapses	Percentage
Treatment A	750	750	100
Treatment B	250	0	0
	1000	750	75

It is correct to say that, of 1,000 cases treated, 75 per cent. relapsed.

Let us repeat the treatments and suppose that the distribution of cases treated now happens to be as follows :—

	Cases treated	Relapses	Percentage
Treatment A	250	250	100
Treatment B	750	0	0
	1000	250	25

It is also correct to say that, of 1,000 cases treated, 25 per cent. only relapsed ; but the figures 25 and 75 have no *real significance.* All that is important that the figures show, is that one treatment was very good, the other very bad.

GRAPH 1.

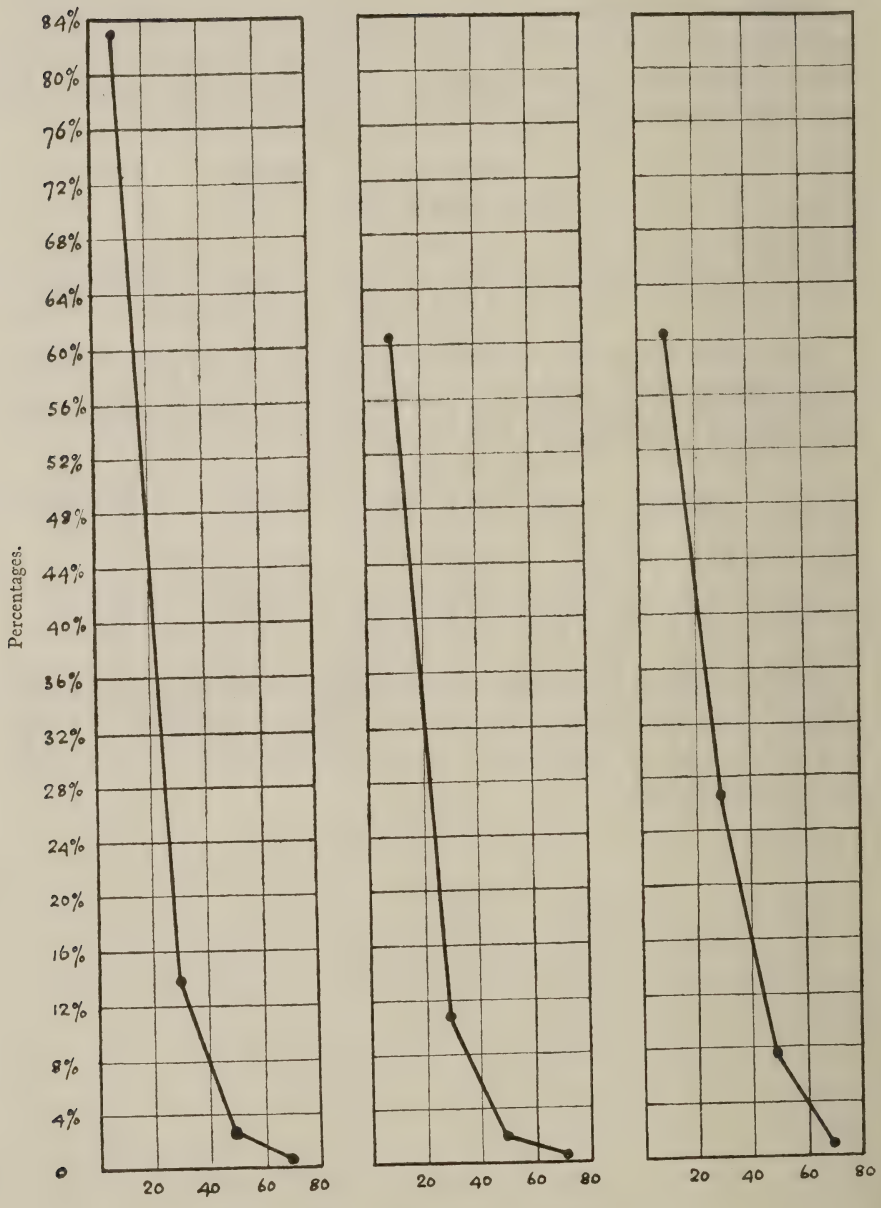
Percentage of total relapses in each 20-day period.

GRAPH 2.

Percentage of cases treated which relapse in each 20-day period.

GRAPH 3.

Percentage of cases treated not having previously relapsed which do so in each 20-day period.



THE TIME AT WHICH RELAPSES OCCUR AFTER CESSATION OF TREATMENT IN SIMPLE TERTIAN MALARIA

The time incidence of relapses can be considered in three ways :—

1. In reference to the *relapses themselves*, i.e., the percentage of the total relapses which occur during each period of time. From an analysis of the time of occurrence of 582 relapses, we found that about four-fifths occur in the first 20 days after treatment, that the majority of the remaining one-fifth occurs in the second 20-day period, i.e., the ratio of the number of relapses in the two periods is about 4 : 1.

2. In reference to the *total cases treated*, i.e., the percentage of cases treated which relapse during each 20-day period of time. Of the cases treated (800), about three-fifths relapse in the first 20-day period, about one-tenth in the second 20-day period : still fewer at later periods, i.e., the ratio of the percentages for the two periods is 6 : 1.

3. In reference to *remainders*, i.e., the incidence among the cases treated less those who have previously relapsed. Of the cases treated (800), about three-fifths relapse in the first 20 days and about one-fourth of ' the remainder ' cases in the second 20-day period. The ratios are here 12 : 5 or 2.4 : 1.

It is possible that, if a large number of cases that had not relapsed in 60 days had been observed for much longer periods, that the values we have given for the first and second 20-day periods would have to be somewhat reduced, but until the actual observations are made, this is purely conjectural.

It must be added that, unless a sufficiently large number of cases are considered, it is not likely that the ratios given above will be observed.

TIME OF ONSET OF THE PAROXYSMS IN SIMPLE TERTIAN MALARIA

From an analysis of 1,000 ' rigors ' or paroxysms, we found that :—

(*a*) Over 90 per cent. of the paroxysms occur during the hours of bodily activity, in our series of cases from 7 a.m. to 6.59 p.m.

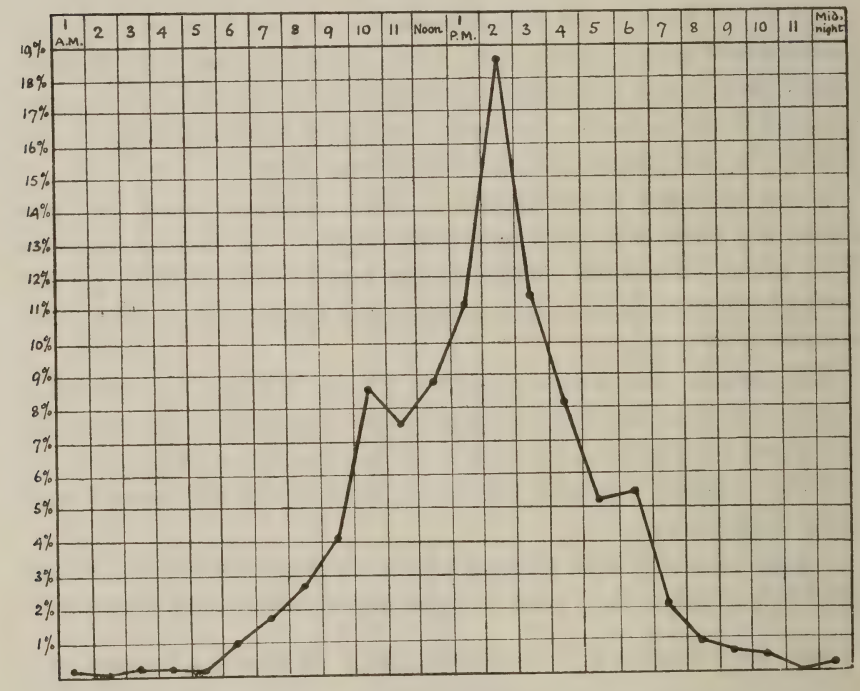
(*b*) The maximum number of paroxysms, about 20 per cent., occurs at 2 p.m.

THE EFFECT OF SEASON ON TREATMENT OF MALARIA

Two series of cases consisting of 76 and 89 patients, respectively, were treated by us at different times of the year with the same treatment, viz., quinine sulphate, grains 90, on two consecutive days only.

In one series the number of relapses was about 40 per cent., in the other, over 90 per cent.

The only factor that we could discover that might account for this difference was that the cases were treated at different times of the year, the good result was obtained in the summer and autumn, the bad result in the winter and spring months.



GRAPH.—Showing the time incidence of 1,000 simple tertian malaria paroxysms; 'Summer' time in operation.

THE MAXIMUM DOSE OF QUININE THAT CAN BE TOLERATED

Quinine sulphate, orally in doses of grains 120 on each of two consecutive days, represents the maximum amount of the drug which can be tolerated by the average case, as the treatment had to be abandoned owing to severe symptoms in five of fifteen cases.

Part II.

TREATMENT OF AN ATTACK

Quinine

(*a*) *Orally.*

Ten grains of quinine sulphate in solution on each of two consecutive days suffice to cut short an attack of simple tertian malaria, and to cause the temporary disappearance of parasites from the cutaneous blood.

While this is so, our routine procedure is to give grains 15, two to three times a day for a few days until the same result is accomplished. The subsequent treatment will be considered later.

(*b*) *Intramuscularly.*

1. *Quinine bihydrochloride.*

Fifteen grains of quinine bihydrochloride in 2 c.c. of water on each of two consecutive days likewise cause the cessation of febrile paroxysms and effect the temporary disappearance of all stages of the parasites from the cutaneous blood. This holds good for *P. vivax* and *P. falciparum.*

2. *Quinine alkaloid.*

Grains 15 to 30 in 1 c.c of sesame oil on each of two consecutive days, has the same effect in cases of simple tertian malaria.

Where the patient can take quinine by the mouth there is usually no necessity for intramuscular injections, but where oral quinine is ineffective, intramuscular quinine remains as a most effective treatment.

(*c*) *Intravenously.*

Quinine bihydrochloride in doses of 10–15 grains in a 10 per cent. solution in normal saline, in one or a series of six injections, causes the cessation of febrile paroxysms and a disappearance of parasites from the cutaneous blood in simple tertian malaria.

In *malignant tertian* malaria these doses do not cause the disappearance of parasites—trophozoites or gametes—from the cutaneous blood.

Arsenic

(*a*) *Organic. Arsenobillon.*

A single intravenous injection, 0.9 gramme, controls the fever, causes the disappearance of *P. vivax* from the cutaneous blood within 24 hours. The same dose has no appreciable effect on the temperature or the parasites in the case of *P. falciparum* or *P. malariæ.*

(*b*) *Inorganic. Liquor arsenicalis.*

In doses of ♏15 daily, failed to control the fever or to cause the disappearance of parasites. In doses of ♏30 daily, the temperature fell to normal within ten days and in 13 of 14 cases parasites disappeared in two to six days.

Silver Arsenic and Antimony

Luargol.

A single intravenous injection of 0.2 gramme, controls the symptoms and causes the disappearance of the parasites in simple tertian malaria.

Antimony

Tartar Emetic.

Intravenous injections of tartar emetic, 2 per cent. solution in one or more doses of 5–15 centigrams, do not control either the rigors or the fever of acute malaria, nor do they cause the disappearance from the blood of any stage of the malaria parasites, whether of *P. vivax* or *P. falciparum.*

Manganese

Collosol manganese 1 c.c. on each of two consecutive days proved to be valueless.

Quitenine and Quinotoxin

The hydrochlorides of these derivatives of quinine proved of no value in the doses used, viz., of about the same amount as that of quinine sulphate which proved effective.

Amylopsin and Trypsin

'Injectio amylopsini' and 'Injectio trypsini' proved to be of no value in the treatment of simple tertian malaria.

Part III.

SUBSEQUENT TREATMENT

We have seen that the immediate effect of quinine and other drugs is to allay the febrile symptoms and to cause the disappearance of parasites, but this condition of apparent cure was, sooner or later, followed by a relapse in the majority of cases. Two questions consequently arose :—

1. The first was, could the condition of apparent cure be *maintained* by continuing the quinine treatment, and if so, how should it be given ?

2. The second was, were these cases in which the administration of quinine was continued for more or less long periods, and which showed no symptoms while taking quinine, really cured ? Would they relapse or not, when treatment was stopped, just as they had done when the treatment had lasted only a few days, or would the number of relapses be now smaller

Question I.

The aspect of the problem that mainly occupied us was, whether if a certain total dose of quinine were given weekly, e.g., grains 30, 60, 90, it were better to administer the quantity on 6 days giving 5, 10, or 15 grains daily, or on two consecutive days only each week, giving 15, 30, or 45 grains daily.

This question was put to the test for a period of eight weeks in a series of cases for each total weekly dose of 30, 60, and 90 grains of quinine sulphate.

An accurate record was kept of the febrile relapses (non-parasitic) and of the parasitic relapses (febrile and afebrile), as determined by the temperature chart and daily blood examinations during the whole of the period.

In each series the record was in favour of the weekly administration of quinine in preference to the daily.

Thus, 30 grains is better administered in the form of two doses of 15 grains, than in the form of six doses of 5 grains.

The best result was obtained by the administration of grains 45 (three doses of grains 15), on each of two consecutive days weekly, this as above stated, giving a better result than grains 15 daily for six days.

An interrupted treatment of 30 grains on each of two consecutive days weekly, also suffices to keep the blood free from trophozoites and to prevent relapses in the majority of cases (while the treatment lasts).

In other words, in order to maintain a patient in a condition of freedom from relapses, an *interrupted* course of quinine is preferable to a *continuous* one.

So far as the actual result was concerned, an equally good one, or nearly so, was obtained in a different way, viz., by giving 15 grains of bihydrochloride intramuscularly on each of the first two days of treatment, and then Liquor arsenicalis ♏30 daily, with two periods of intermission for eight weeks (two weeks on, one week off, two weeks on, one week off, two weeks on).

The comparative figures for this and the previous interrupted quinine treatment are the following :—

	Quinine injections, two only, followed by Liq. arsenicalis ♏30 daily	Quinine sulphate Gr. 45 on two consecutive days weekly for 8 weeks
Percentage of parasitic febrile relapse cases per cases treated (average per week)	2·7	1·8
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated (average per week	8·7	10·3
Number of cases treated...	33	74

What we have just considered is a method of maintaining freedom from relapses *while the treatment is in force.* We shall now consider a different question, viz. :—

QUESTION 2.

This question resolves itself into an enquiry as to whether by any course of treatment, short or long, a curative effect would be obtained, i.e., freedom from relapses after cessation of treatment, over an observation period of sixty days (or longer).

Many methods were tried, but in nearly all, when treatment was stopped, the number of relapses was large, and there is at present no method known which will cure all cases, even if the treatment lasts eight weeks.

Many methods of cure continue however, to be advocated, but they are not supported by trustworthy evidence, more especially in regard to an adequate observation period.

The following two treatments gave us the best results :—

	Number of cases treated	Number of cases not relapsing but lost sight of before the expiration of 60 days	Number of cases relapsing in an observation period of 60 days	Relapses per cent. min.	Relapses per cent. max.	Time of Year.
Liquor arsenicalis minims 30 daily with 1 or 2 periods of intermission with an injection of quinine bihydrochloride on each of the first two days only	32	—	4	12·5	12·5	End of treatment August :—1 case ; September :—17 cases ; October :—14 cases NOTE.—One additional case was not controlled by the treatment.
Novarsenobillon 0·9 grm. intravenously on the 1st, 8th, and 15th days with quinine bihydrochloride grs. 15 intramuscularly on the 1st and 2nd, 8th and 9th, and 16th days.	12	1	—	8·3	16·6	End of treatment December :—12 cases

It is worthy of note that a treatment which is ' good ' whilst it lasts is not necessarily followed by a ' good ' result when it has ceased. Thus the treatment noted above, viz., grains 45 × 2 weekly for eight weeks, while ' excellent ' while it lasted, was followed by 80 per cent. of relapses when the treatment had finished.

Whereas the arsenic treatment also a good one while it lasted, was followed by a ' good ' result also when it had ceased.

THE DISAPPEARANCE OF MALIGNANT TERTIAN GAMETES (CRESCENTS) UNDER QUININE TREATMENT

1. With a dose of grains 30 or 45 daily, crescents do not persist in the blood in the majority of cases for more than three weeks. Whether they would disappear equally rapidly without quinine we did not determine.

2. Similarly with quinine sulphate grains 30 on each of two consecutive days weekly for five weeks, the crescents diminished from 50 per cent. in the first week to 6 per cent. in the fifth week of treatment.

APPENDIX

The Titles, number of volume and date of Publication of the Studies in the Treatment of Malaria (I–XXXI) are given below :—

Annals of Tropical Medicine and Parasitology.

I Intravenous Injections of Tartar Emetic. Vol. XI, p. 91. 1917.

II Intramuscular Injections of Quinine Bihydrochloride in Simple Tertian Malaria. Vol. XI, p. 113. 1917.

III Intravenous Injections of Quinine Bihydrochloride. Vol. XI, p. 149. 1917.

IV Intramuscular Injections of Anylopsin and Trypsin in Simple Tertian Malaria. Vol. XI, p. 165. 1917.

V Intramuscular Injections of Quinine Alkaloid in Simple Tertian Malaria. Vol. XI, p. 173. 1917.

VI Oral Administration of Quinine for Two Consecutive Days only in Simple Tertian Malaria. Vol. XI, p. 283. 1918.

VII Oral Administration of Quinine Sulphate daily over Prolonged Periods in Simple Tertian Malaria. Vol. XI, p. 309. 1918.

VIII Oral Administration of Quinine Sulphate for Two Consecutive Days Weekly over Prolonged Periods in Simple Tertian Malaria. Vol. XI, p. 331. 1918.

IX A Comparison of the Results of Interrupted and Continuous Quinine Treatment. Vol. XI, p. 359. 1918.

X Oral Administration of Quinine Sulphate Grains 120 on Two Consecutive Days only in Simple Tertian. Vol. XI, p. 417. 1918.

XI Oral Administration of Quinine Sulphate Grains 90 on Two Consecutive Days Weekly over a Period of Three Weeks in Simple Tertian Malaria. Vol. XI, p. 421. 1918.

XII At what Time after Cessation of Quinine Treatment do Relapses occur in Simple Tertian Malaria ? Vol. XI, p. 425. 1918.

XIII Oral Administration of Quinine Sulphate Grains 90 on Two Consecutive Days only in Simple Tertian Malaria (Second Series). Vol. XII, p. 71. 1918.

XIV Quinine Bihydrochloride Grains 30 intramuscularly, and Quinine Hydrochloride Grains 30 orally, Daily for 12 days, in Simple Tertian Malaria Vol. XII, p. 197. 1918.

XV A Factor hitherto overlooked in the Estimation of the Curative Value of Treatments of Malaria. Vol. XII, p. 201. 1918.

XVI Intravenous Injections of Novarsenobillon in Simple Tertian Malaria. Vol. XII, p. 211. 1918.

XVII Oral Administration of Quinotoxin for two Consecutive Days only, in Simple Tertian Malaria. Vol. XII, p. 217. 1918.

XVIII. A Comparison of the Value of Continuous and Interrupted Quinine Administration in Simple Tertian Malaria (Second Communication). Vol. XII, p. 303. 1919.

XIX Intravenous Injections of Disodoluargol in Simple Tertian Malaria. Vol. XII, p. 339. 1919.

XX Intramuscular Injections of Collosol Manganese in Simple Tertian Malaria. Vol. XII, p. 345, 1919.

XXI Arsenic in Simple Tertian Malaria. Vol. XII, p. 371. 1919.

XXII Intramuscular Injections of Quinine Bihydrochloride Grains 15 on each of two Consecutive Days only, in Malignant Tertian Malaria. Vol. XIII, p. 63. 1919.

XXIII Oral Administration of Quinine Sulphate Grains 30 on each of two Consecutive Days weekly, over a Period of Five Weeks in Malignant Tertian Malaria. Vol. XIII, p. 69. 1919.

XXIV The Disappearance of Crescents under Quinine Treatment. Vol. XIII, p. 73. 1919

XXV Arsenic in Malignant Tertian Malaria. Vol. XIII, p. 75. 1919.

XXVI The Action of Arsenic and of Quinine on Quartan Malaria. Vol. XIII, p. 97. 1919.

XXVII Intravenous Injections of Novarsenobillon and Intramuscular Injections of Quinine Bihydrochloride in Simple Tertian Malaria. Vol. XIII, p. 101. 1919.

XXVIII Quitenine Hydrochloride in Simple Tertian Malaria. Vol. XIII, p. 117. 1919.

XXIX Oral Administration of Liquor Arsenicalis Minims 30 daily for 16 Days with Quinine Bihydrochloride Grains 15 Intramuscularly on the 1st and 2nd, 8th and 9th, 15th and 16th days, in Simple Tertian Malaria. Vol. XIII, p. 119. 1919.

XXX At what time after Cessation of Quinine Treatment do Relapses occur in Simple Tertian Malaria? (Second Communication). Vol. XIII, p. 125. 1919.

XXXI The Time of Onset of the Paroxysms in Simple Tertian Malaria. Vol. XIV, p. 365. 1921.

NOTES ON AUSTRALIAN CESTODES

BY
P. A. MAPLESTONE
AND
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(*Received for publication* 3 *July*, 1923)

No. VII.

In this paper, which is the last of the series, three new species and one new genus are described. Further information relating to the morphological characters of *Monopylidium macracanthum* and *Linstowia echidnae* are also included.

Bothridium ornatum, n. sp.

On several occasions specimens of this worm were obtained from Carpet Snakes (*Python spilotes* var. *variegatus*, Gray), taken in the Townsville district.

External Anatomy.

The largest worm measured about 65 cm. in length, and the greatest breadth was 7 mm.

Head. The head measures about 4 mm. in breadth and 5 mm. in length. It consists of two cylindrical muscular tubes, one lying dorsally and the other ventrally. They are attached to each other throughout their whole length by a broad membrane. They are funnel-shaped and are open at both ends, the posterior opening being the smaller and directed inwardly (fig. 1).

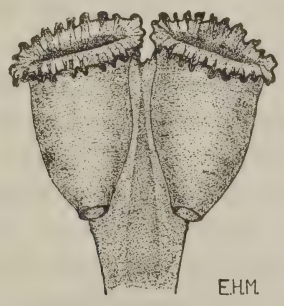

Fig. 1. *Bothridium ornatum*, n.sp. Head. × 9.

Unlike *B. pithonis*, the anterior opening is surrounded by a very conspicuous fleshy frill.

Segments. These are very numerous, and are all broader than long. The lateral borders are imbricated.

INTERNAL ANATOMY.

No points of difference could be observed between this worm and *B. pithonis*, as described by Braun (1900).

DIAGNOSIS.

We have compared our specimens with the literature of all the known species of *Bothridium* and find that it differs from them in the characters of the head, viz., the possession of a fleshy frill round the anterior openings. It is proposed to name it *Bothridium ornatum*, n. sp.

Type specimens are in the Museum of the Liverpool School of Tropical Medicine.

Monopylidium fieldingi, sp. nov.

These cestodes were found in the intestine of several specimens of the Butcher bird (*Cracticus destructor*, Temm.), all of which were shot in the neighbourhood of Townsville, North Queensland.

EXTERNAL ANATOMY.

A complete specimen of the worm was not available for examination, so the total length cannot be given, but, from the appearance of several fragments taken together, and the rate of development, it is estimated that a complete adult would be over 50 mm. in length. The maximum breadth attained is 1·2 mm.

Head. The rostellum is strongly retracted in all the scolices available for study, with the result that anteriorly, it is in the shape of a truncated cone. The rostellum apparently invaginates when in this state, so that the tip is in the form of a saucer-shaped depression, around the edge of which is a double crown of alternating hooks (fig. 2). The hooks are about forty in number in each row and are of a definite rose-thorn shape when seen in profile; when viewed dorso-ventrally they present a Y-shaped appearance, the handle of which is long and the limbs of unequal length. They measure about 22μ in length (fig. 3).

The scolex reaches its maximum breadth (about 0·4 mm.) across the posterior borders of the suckers. These organs, when viewed in profile, are seen to stand out slightly from the surface. They are circular in shape, and measure about 130μ in diameter. They look outwards and slightly forwards, and are unarmed.

Immediately behind the suckers the scolex narrows slightly, and its termination is marked by a somewhat indefinite constriction (probably an artifact), which lies about 0·4 mm. from the anterior end. Immediately behind this constriction is an unsegmented portion about 0·4 mm. in length and 0·25 mm. in breadth. At this point, *i.e.*, about 0·8 mm. from the anterior extremity, the first traces of segmentation appear.

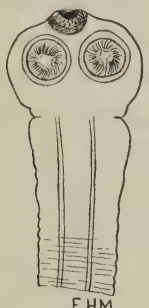

FIG. 2. *Monopylidium fieldingi*, n.sp. Head. × 35.

FIG. 3. *Monopylidium fieldingi*, n.sp. Hook, highly magnified.

Segments. Segmentation soon becomes quite distinct, and the proglottides are seen to have their lateral borders curved, with the convexity outwards, and the postero-lateral angles projecting fairly widely. After about the fiftieth proglottis the length increases slightly more rapidly than the breadth, so that the proportion of breadth to length alters somewhat, but never to such an extent that the length becomes greater than the breadth.

Mature proglottides measure 0·17 mm. antero-posteriorly, 0·4 mm. across the anterior, and 0·47 mm. across the posterior border. The medullary portion at this stage is only 0·170 mm. in breadth.

INTERNAL ANATOMY.

The longitudinal muscle consists of relatively thick fibres, which are disposed in two layers, but, as the material was not in a good

enough state of preservation, and was also somewhat scanty, sections could not be cut, and therefore a detailed description of the musculature cannot be given.

Nervous system. The details of this system were not investigated.

Excretory system. The longitudinal excretory vessels lie at a considerable distance from the lateral borders, so that the medulla is correspondingly narrow. The ventral vessels are uniform in diameter throughout their whole length; they measure about 20μ in optical section. The narrower dorsal vessel lies directly above the ventral, and the ducts from the reproductive organs pass between them.

Genitalia. The genitalia develop slowly, so that there are about one hundred segments showing traces of the sexual organs before they become sufficiently developed to be clearly distinguished.

Testes. The testes are circular or slightly oval, and number about sixteen to twenty-one in each segment. When viewed dorso-ventrally they are seen to occupy the space posterior to the female glands, but on each side a few follicles are on a level with the vitellarium, or even with the ovary itself. The vasa efferentia unite into a vas deferens, which is thrown into several coils in front of the right lobe of the ovary. There is no external vesicula seminalis, and the vas deferens passes directly into the base of the cirrus pouch. The cirrus pouch is relatively long and narrow, its dimensions being 130μ in length and 45μ in breadth. Beginning mesial to the excretory vessels, it runs towards the right side in all cases, and very slightly posteriorly, and, passing between them, opens in a small atrium, which in turn opens on the right lateral border, about the junction of the anterior and middle thirds. The characters of the cirrus could not be made out (fig. 4).

Receptaculum and vagina. The vagina is a long straight tube which commences at the genital pore, immediately posterior to the opening of the cirrus. From here it runs transversely inwards, thus diverging more and more from the cirrus pouch as it goes; it passes dorsal to the right lobe of the ovary, dilating over the ovarian duct into a small but distinct receptaculum seminis.

Ovary. The ovary is centrally situated in the anterior half of the medulla. It is approximately bilaterally symmetrical and

consists of three lobes, two pointing laterally and a median lobe pointing anteriorly (fig. 4).

Uterus. The uterus develops as a uniform sac devoid of out-pocketings. It eventually fills the entire segment antero-posteriorly and extends laterally to the excretory canals.

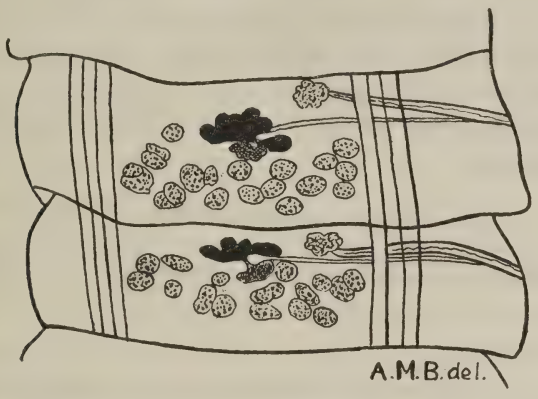

FIG. 4. *Monopylidium fieldingi*, n.sp. Ripe segment, showing genitalia. × 69.

It is split up into capsules having a reticular form, each capsule containing up to about twelve eggs. Later, a separate capsule appears to be formed around each egg. The uterus was not fully

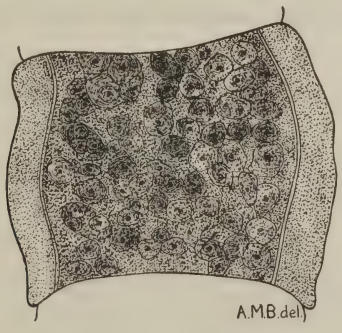

FIG. 5. *Monopylidium fieldingi*, n.sp. Gravid segment. ×28.

matured in any specimen, therefore the nature and extent of these capsules could not be determined (fig. 5).

Eggs. No ripe eggs were seen.

DIAGNOSIS.

As this worm possesses all the characters given by Fuhrmann (1899) in the diagnosis of *Monopylidium*, there is no doubt that it belongs to this genus.

As it disagrees with all known species of *Monopylidium*, it is consequently new and accordingly named *Monopylidium fieldingi* after Mr. J. W. Fielding, Senior Assistant at the Australian Institute of Tropical Medicine, who collected this and most of the other material described in this series.

Type specimens are in the Museum of the Liverpool School of Tropical Medicine.

Monopylidium macracanthum, Fuhrm.

This worm was found on several occasions in the intestine of the spur-winged plover (*Lobivanellus lobatus*, Lath.).

EXTERNAL ANATOMY.

Fixed specimens measure about 45 mm. in length, and 1·8 mm. in breadth, but there is apparently some shrinkage from fixation, so that these dimensions will have to be revised when fresh material is available.

There is no trace of a 'neck', the scolex passing directly into the segmented chain.

Head. The scolex is about 0·6 mm. in breadth, and is conical anteriorly (fig. 6). The suckers are relatively small, but well

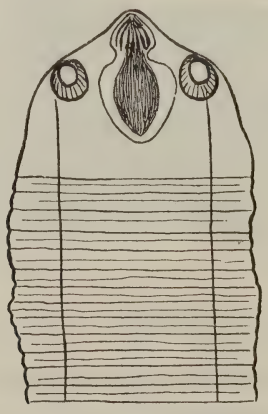

FIG. 6. *Monopylidium macracanthum*, Fuhr. Head. × 35.

developed; they measure 0·13 mm. in diameter, and are situated on the scolex just where it begins to narrow anteriorly. They face forwards and slightly outwards. The tip of the scolex bears a rostellum, which was only seen in the retracted state in all the specimens available for examination.

Rostellum. The rostellum is a muscular organ, and completely fills the fossa into which it is contracted; this fossa measures about 0·47 mm. in depth and 0·24 mm. in greatest breadth, being oval in shape. The rostellum is seen to consist of two parts, a small anterior conical part and a larger oval posterior portion. The anterior part is distinctly marked off from the posterior by a neck-like constriction. It is armed with a double row of relatively large hooks, which number twenty-six (? twenty-eight). They are of two sizes, alternating, the larger measure 145μ and the smaller about 110μ. They have a long dorsal root and blade, and a short ventral root, 145μ (fig. 6).

The posterior portion of the rostellum is oval in optical section, and, in the contracted state, appears very muscular.

Segments. The dimensions of sexually mature segments are about 1 mm. across the anterior, and 1·2 mm. across the posterior borders, so that the postero-lateral angles are only slightly projecting; their length is 0·4 mm., giving a proportion of breadth to length of approximately three to one (fig. 7).

Internal Anatomy.

Muscular system. The cuticle is thickly studded with calcareous corpuscles, and the muscle layers are only thinly developed, but their exact disposition cannot be given as there was not sufficient material from which to cut sections.

Nervous system. This was not carefully investigated. It was noted, however, that a single nerve ran external to the excretory vessels.

Excretory system. The lateral excretory vessels are situated directly one above the other, the dorsal being the narrower. The ventral vessels are joined by a commisural channel, which runs across immediately posterior to the testes.

Genitalia. The reproductive organs can first be made out in about the tenth proglottis, and from here on they steadily become more distinct, until they reach maturity. There are about fifty

proglottides sexually mature as far as microscopic characters go, before the uterus becomes apparent.

The genital pores are circular, relatively large and irregularly alternating.

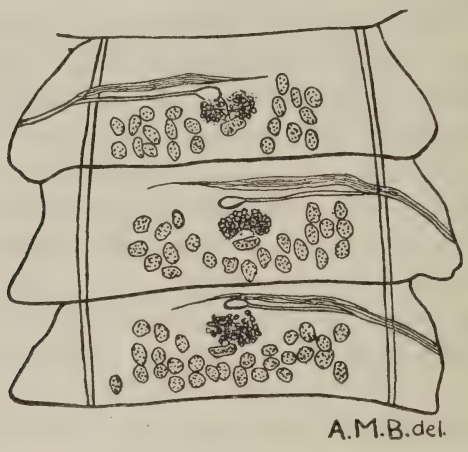

Fig. 7. *Monopylidium macracanthum*, Fuhr. Segments showing genitalia. × 35.

Testes. The testes number twenty-three to thirty in each segment, and they occupy the usual dorsal position lying on each side of, and posterior to, the ovary when viewed from above (fig. 7).

Vas deferens. The various vasa efferentia pass forwards and unite in front of the ovary into a many-coiled vas deferens, which apparently fulfils the function of a vesicula seminalis, as there is no indication of this organ otherwise. These coils lie transversely on the pore side about the junction of the anterior and middle thirds of the segment, and run direct into the mesial end of the cirrus sac. The cirrus pouch is long and thin, and contains a few coils of vas deferens and the cirrus. In its course it runs laterally between the excretory vessels, and, passing slightly posteriorly, opens about the middle of the lateral border of the segment. The characters of the cirrus could not be clearly determined, as it was in all cases entirely within the pouch.

Ovary. The ovaries consist of two equal lobes placed one on each side of the mid-line, and each is composed of many subsidiary branches (fig. 7). The anterior border of the ovary is about the

level of the mid-transverse plane of the segment. The lobes each measure about 80μ in diameter. A duct runs inwards from each lobe and they unite in the mid-line to form the oviduct which joins the fertilisation canal running to the uterus. From this junction a duct passes directly backwards to the vitellarium, and it is surrounded for a little part of its length by the small compact shell gland.

Receptaculum and vagina. The vagina opens from the genital atrium, ventral and posterior to the male opening. It is a straight tube which at first follows the posterior border of the cirrus pouch as it passes mesially, but it soon leaves this and runs directly inwards towards the anterior border of the ovary, where it dilates in front of the lobe on the pore side, into a small receptaculum seminis; from the mesial end of the receptaculum a duct passes posteriorly to join with the two branches of the ovarian duct (one from each lobe), where they unite.

Vitelline glands. The vitellarium lies behind the ovary in the mid-line. It is a compact body, showing no trace of branching, but is somewhat indefinitely divided into right and left lobes, with the result that the whole organ is more or less kidney-shaped, with the 'hilum' facing forwards. A duct runs from its centre anteriorly, to join the fertilisation canal.

Uterus and eggs. The uterus is at first simple, and saccular, but later it splits up into capsules, each containing a single oncosphere. The eggs are circular or slightly oval, being about 80μ in diameter; the contained embryo measures about 30μ.

The male copulatory organs persist and are quite distinct even in fully gravid segments, long after all the other reproductive organs have entirely disappeared.

DIAGNOSIS.

The presence of a double crown of hooks, identical in size with those of *Monopylidium macracanthum*, Fuhrmann, together with the uterus split up into capsules each containing one oncosphere, leaves no room for doubt that this species is *M. macracanthum*, Fuhrmann. The only point in which it differs is in the number of hooks. Fuhrmann gives twenty-two, whereas in the present species at least twenty-six were seen clearly. As hooks easily become

detached, and further, as their number often varies, no importance can be placed on this difference.

Fuhrmann (1907) originally recorded this parasite from *Helodroncus octropus* in Africa and India, and as his description is somewhat meagre, it was thought desirable to amplify his account when making this new record of the worm in a fresh host and locality, viz., *Lobivanellus lobatus*, from North Queensland.

Type specimens of this cestode were placed in the Museum of the Liverpool School of Tropical Medicine.

Linstowia echidnae, Thompson (1893).

D'Arcy Thompson (1893) described a cestode from the Echidna from Australia. In his brief description he mentions that the worms were very contracted.

We have a large collection of immature worms from the same host which, as far as can be ascertained, are the same species. Our material, however, is not so strongly contracted as that of Thompson, and accordingly the condition of the scolex in particular is somewhat different.

As Thompson's description is rather incomplete, the following additional particulars are given.

Head. The anterior surface of the scolex is quite devoid of a rostellum, in fact in some cases it has a slight central depression.

The dimensions of the rounded scolex differ slightly in different specimens, varying between 0·76 mm. and 0·58 mm. in breadth. The maximum diameter is just posterior to the suckers.

Suckers. The four suckers are placed well forward on the scolex; they are well developed, circular organs, lying flat on the surface, and their openings look outwards and slightly forwards (fig. 8).

Segments. At first the proglottides are almost rectangular in shape, broader than long, with no projection of the postero-lateral angles; but as development advances the posterior angles come to project somewhat, with the result that the anterior borders of the segments are shorter than the posterior. The dimensions of the most fully developed segments available for study are 1·6 mm. across the posterior borders, and about 1·35 mm. across the anterior, with a length of about 0·45 mm., being approximately a proportion of breadth to length of three to one. The posterior border is slightly

curved, with the convexity backwards, and to some extent it overlaps the succeeding segment. The cuticle of the worm is thrown into several slightly marked longitudinal folds, which on the

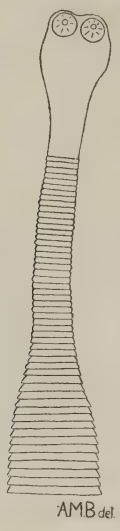

Fig. 8. *Linstowia echidnae* (Thompson). Head and anterior segments. × 12.

posterior free borders of the segments give an appearance of scalloping (figs. 8 and 10).

Internal Anatomy.

Muscular system. Transverse sections show a relatively thick cuticle and cortical parenchyma, and the longitudinal muscle is disposed in two layers, completely encircling the segment, the outer layer being slightly the thicker of the two (fig. 9).

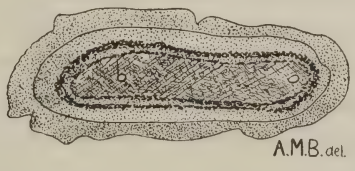

Fig. 9. *Linstowia echidnae* (Thompson). Transverse section showing musculature. × 35.

Nervous system. This system was not investigated.

Excretory system. The dorsal excretory vessel is narrower than the ventral, and lies to the outer side of the latter. They both pass dorsal to the ducts of the male and female organs.

Genitalia. The genital pores cannot be made out, as in no instance is development complete enough to show them, but from the direction of the immature sex ducts they would probably open about the centre of the lateral border; they are irregularly alternating, there being, as a rule, three or four on one side followed by about the same number on the other side. The reproductive organs are single in each proglottis (fig. 10).

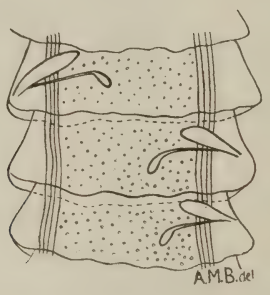

Fig. 10. *Linstowia echidnae* (Thompson). Segments showing cirrus pouch and vagina. × 35.

Testes. Owing to the immature condition of the worms, only traces of the testes can be distinguished. They are numerous and are scattered dorsally across the whole width of the medulla.

Vas deferens. No details of this organ can be made out, but it is seen entering the mesial end of the developing cirrus pouch. At the most advanced stage of development observed, the cirrus pouch is represented by a relatively long, straight, tubular structure, which runs inwards and slightly forwards from opposite the centre of the lateral border, so that it lies across the antero-lateral angle of the segment on the side on which it will eventually open.

Ovary. This organ lies slightly to the pore side of the median line, and about midway between the anterior and posterior borders of the segment. No details of its structure can be given because it is quite immature.

Receptaculum and vagina. The vagina is seen as a straight tube, running inwards along the posterior border of the cirrus pouch, which it leaves about its centre, and running directly inwards, ends in a small expansion, evidently the beginnings of a receptaculum seminis, around which the developing female genitalia can be seen.

On account of the undeveloped condition of the worms, no particulars of the vitelline glands, shell glands, uterus or eggs can be given.

Paramoniezia suis, n.g., n. sp.

One specimen was obtained from the intestine of a wild pig (*Sus scrofula*), near Townsville, North Queensland.

EXTERNAL ANATOMY.

The worm is lancet-shaped and measures about 12 cms. in length and 10 mm. maximum breadth.

Head. The head is very small and measures only about 300μ in breadth. It is unarmed and there is no rostellum. The suckers are extremely small; they were too shrunken to give accurate dimensions. There is no neck.

Segments. These are always broader than long and their free edges are imbricated. A typical mature segment measures 200μ in length and 9 mm. breadth. The genital pores are double.

INTERNAL ANATOMY.

Muscular system. The longitudinal muscle fibres are arranged in a single layer, composed of numerous bundles measuring about 60μ thick. External to this is a thin layer of circular fibres. A few dorso-ventral fibres also occur.

Nervous system. This system could not be investigated because even the main lateral nerve could only be seen with difficulty.

Excretory system. The ventral excretory vessel is very large, and in most transverse sections it appears to occupy the whole of the lateral dorso-ventral space. The diameter of the tube is about 150μ.

The large size of this vessel, and the numerous branches to which it gives rise, made it difficult to determine whether a dorsal vessel was present or not; but careful examination led us to the conclusion that a dorsal vessel was absent.

Genitalia.

Testes. These are very numerous (at least three hundred). They extend on each side almost to the lateral extremity of the segment and are not grouped round the ovary, but extend right

across the segment. Each testis measures about 65μ by 45μ. Antero-posteriorly they lie in four or five rows, and dorso-ventrally in from one to three layers.

Vas deferens. The vas deferens on each side runs dorsal to the ventral excretory vessels. The cirrus pouch is tubular and lies lateral to the water vessel. Its median portion contains an internal vesicula seminalis. No external vesicula seminalis was seen. The cirrus is unarmed.

Ovary. This organ is paired in each segment, and is of the usual *Cittotaenia* or *Moniezia* type.

Receptaculum and vagina. From the pore the vagina runs inwards, having at first a diameter of about 60μ; it expands immediately internal to the excretory vessels into a large transversely elongated muscular sac (the receptaculum seminis) measuring about 650μ in length and 150μ breadth. Its median extremity, which lies close to the ovary, is continued as a short coiled narrow tube to the fertilization canal.

A most important point is the fact that whilst the vagina is always ventral to the cirrus pouch on right side, it may be either dorsal or ventral on the opposite side.

Uterus. This is first apparent as a cell-string running across the segment. It develops into a tube, devoid of outgrowths, and extends on each side to the extreme edge of the segment.

Eggs. The ripe egg has a diameter of 45μ; the outer shell has a double contour. The hexacanth embryo measures about 24μ, and a pyriform apparatus is entirely absent. Between the embryo and the shell a small quantity of yolk can be seen.

DIAGNOSIS.

This worm obviously belongs to the family *Anoplocephalidae*, Fuhrm., 1907, and the sub-family *Anoplocephalinae*, Blanchard, 1891. The only two genera within this sub-family possessing double genital pores and a single cirrus pouch on each side are *Cittotaenia*, Rheim., 1881, and *Moniezia*, Blanchard, 1891, and these differ from each other in one particular only, viz., in *Cittotaenia* the vagina is ventral to the cirrus pouch on both sides, whilst in *Moniezia* the vagina is ventral to the cirrus pouch on the right side and dorsal on the left. In the present species the relationship of the cirrus to vagina is variable, the vagina being

sometimes dorsal and sometimes ventral to the cirrus on the left side, in the same strobila.

It is necessary, therefore, to erect a new genus for this species, which we have named *Paramoniezia suis*, n.g., n. sp.

The characters of the new genus are as follows:—*Paramoniezia*. With the characters of the genus *Moniezia*, except that on the left side the cirrus is sometimes dorsal and sometimes ventral to the vagina.

Type specimens are in the Museum of the Liverpool School of Tropical Medicine.

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A NOTE ON *OPHIOTAENIA PUNICA* (CHOLODOVSKI, 1908), LA RUE, 1911

BY
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AND
S. ADLER

(*Received for publication* 3 *July*, 1923)

Two specimens, both gravid, were obtained from the intestine of *Causus rhombeatus*, in Freetown, Sierra Leone.

EXTERNAL ANATOMY.

The larger complete specimen measured 9 cms. in length, and the maximum breadth was 3·3 mms.

Head. The head is almost square and measures 1·5 mm. broad; it is unarmed.

Suckers. The four suckers have a diameter of 0·67 mm. The neck is 0·7 mm. long.

The worm is made up of about one hundred and forty segments. The first proglottides are broader than long; they gradually lengthen towards the posterior, the last proglottis being 4 mm. long and 2 mm. broad.

The genital pores are irregularly alternate, and open at the middle of the lateral border.

INTERNAL ANATOMY.

Musculature. The musculature consists of a series of (1) small subcuticular fibres, situated immediately beneath the cuticle, (2) a double layer of longitudinal muscles which are not strongly developed, (3) a few diagonal fibres, and (4) circular fibres which are very scanty.

Excretory system. There are two water vessels on each side, the ventral vessel being much larger than the dorsal vessel.

Nervous system. A single nerve is present on each side, lying lateral to the water vessels. The parenchyma is strongly developed.

Genitalia. The testes are confined to the lateral fields in front of the ovary, and median to the vitellaria. There are from one hundred and seventy to two hundred and thirty in each segment; they are oval in shape, their long axes being horizontal (fig. 1).

Vas deferens. The cirrus pouch first becomes evident about 15 mm. behind the head; it lies either anterior or posterior to the vagina and extends beyond the vitellaria, being up to 670μ in length (fig. 1).

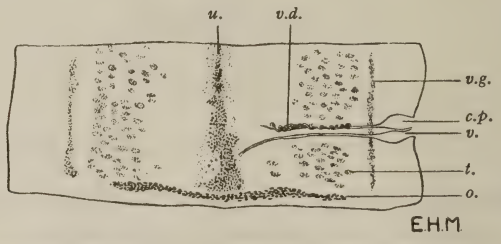

FIG. 1. *Ophiotaenia punica.* A ripe segment, shewing genitalia. *v.g.*—Vitelline glands. *c.p.*—Cirrus pouch. *v.*—vagina. *t.*—testes. *o.*—Ovary. *v.d.*—Vas deferens. *u.*—Uterus. × 35.

The cirrus is spiny and is continuous with an internal seminal vesicle, which latter occupies about two-thirds of the cirrus pouch. The vas deferens lying outside the pouch is coiled.

Ovary. The ovary is long and narrow and is not bilobed (fig. 1); it is situated posteriorly.

Vagina. The vagina lies either anterior or posterior to the cirrus pouch. It runs almost straight towards the middle of the segment, and then turns posteriorly (fig. 1).

Vitellaria. The vitellaria are lateral, and consist of small acini measuring about 30μ to 36μ in diameter (fig. 1).

Uterus. The uterus is a straight tube running antero-posteriorly; in mature segments it has from eight to twelve lateral pouches on each side. There is a small shell gland situated immediately behind the middle of the ovary (fig. 2). In transverse

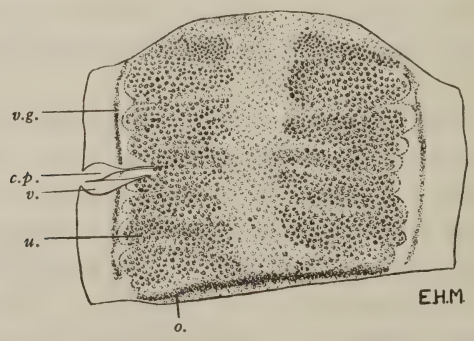

FIG. 2. *Ophiotaenia punica.* A segment shewing gravid uterus. *v.g.*—Vitelline glands. *c.p.*—Cirrus pouch. *v.*—Vagina. *u.*—Uterus. *o.*—Ovary. × 35.

sections of segments in which the uterus was gravid no uterine pores were seen.

Eggs. The eggs are 30μ in diameter, and in appearance resemble the eggs of *Hymenolepis nana.* The oncosphere is from 13μ to 15μ in diameter. The embryophore has a thickness of about 3μ (fig. 3).

Fig. 3. *Ophiotaenia punica.* Egg. × 733.

Diagnosis. Ophiotaenia punica was first found in a dog in Tunis by Cholodovski (1908), but, owing to its morphological characters, Hall, Ransom and La Rue thought the true host was a snake. They presumed that the dog had eaten a snake. Southwell (1922) recorded this parasite from *Paradoxurus hermaphroditicus* (Malayan palm civet) in Calcutta.

This is the first definite record of *Ophiotaenia punica* from a snake.

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A PYRRHOCORID BUG CAPABLE OF BITING MAN

BY

B. BLACKLOCK, M.D.

From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone

(*Received for publication* 4 *July*, 1923)

So far as I am aware, the following notes contain the first record of the fact that a bug of the family *Pyrrhocoridae* has been found to bite man. The observation was made at Freetown, Sierra Leone, but so near to the time of my departure that little opportunity of carrying out experimental work on the subject was available.

The natural order Rhynchota or Hemiptera is divided into the sub-orders Heteroptera and Homoptera; the Heteroptera into Gymnocerata and Cryptocerata; the Gymnocerata into several families, of which the family Pyrrhocoridae is one; in a sub-family of this—the Pyrrhocorinae—occur many genera, to one of which, namely, Dysdercus, belongs the insect with which I am here dealing. Mr. Lang, of the British Museum, has kindly identified the species for me as *D. superstitiosus*, F.

Dysdercus superstitiosus was observed by me at Freetown, in 1921, specimens being found even indoors in the laboratory. At that time I made a few experiments in order to see if the insects would bite; the bugs were placed singly in wide test-tubes and applied to the arm, but they showed no inclination to bite or even stay on the skin, making, on the contrary, efforts to escape by climbing up the test-tubes; it was concluded, rather prematurely as it now appears, that they were entirely non-biting in so far as human beings are concerned. They were observed in numbers on the ground, especially in the vicinity of a silk cotton tree, *Eriodendron anfractuosum*, situated about a hundred yards from the laboratory. It was observed that they appear to see very well, as they are extremely sensitive to any movement made in their vicinity; also that they make off instantly and move away with

great rapidity when disturbed. This year, in April, the silk cotton tree pods were opening on the tree and it happened that on one or two days the wind drove the cotton along, in the direction of the ground on which the laboratory stands. In the flocculi of silk cotton which were wafted on to the ground were seen numerous small red bugs, which in most cases extricated themselves quickly and ran about actively. Occasionally a silk cotton seed was carried along with the floating fragments of fibre, and it was not unusual to see on the ground a seed covered with bugs, some of them with their beaks inserted into it and others trying to pierce it, the seed being pulled in all directions during the process. When the bugs grew larger some were observed to develop cannibal habits, more especially when placed together in test-tubes with no food.

On the 24th of April, 1923, while sitting out in front of the laboratory just after sunset I experienced a sharp bite on the front of the ankle. On looking to see what was biting I moved slightly and could observe no mosquito or other biting insect, but saw a red bug moving rapidly off my sock. It was not possible to be sure that the definitely painful bite was caused by the bug; the reaction was a small itching swelling which had disappeared in twenty-four hours. Two days later, on the 26th April, at the same place and time, a bite was again felt on the same ankle. Leaning forward carefully I saw a red bug biting busily through my thick black sock. The bug's body, as its beak went deeper and deeper into the skin, assumed an attitude which was nearly vertical; considerable irritation was felt at intervals during the time the biting was going on. The process was timed and had lasted nearly four minutes when the bug was disturbed by a large black ant, which, in passing rapidly across the ankle, collided with the bug. The latter instantly made off and was escaping when it was captured in a test-tube which had been kept at hand since the previous observation, so as to be ready should the opportunity arise again. The reaction was on this occasion also only local, but was much more definite. Itching and irritation were felt, and in an hour's time a circular swelling of the size of a sixpenny piece had developed, well raised in the centre. This remained and in two days was rather hard, but it went away gradually in about five days from the time of the bite. The bug was examined and found to be a last larval stage:

the dissection of it did not reveal the presence of blood in the alimentary canal, nor were flagellates found; but the dissection had to be carried out with the light of an oil lamp, which was unsatisfactory.

Laboratory experiments. Several experiments were carried out by placing single bugs in large test-tubes on the human skin, but in no case was biting observed, the bugs being only anxious to escape by climbing up the tubes. Too much stress should not be laid upon the negative results of these experiments, in view of the fact that bugs which had been seen attacking the seed of the silk cotton tree on the ground made no attempt to attack the seeds when placed with them in tubes similar to those used for the skin experiments. Ballou (1906) made a comparable observation. He says: 'Although the cotton stainers are known to feed on the ripe cotton seed about the gin-houses, they would not do this in the laboratory, nor would they feed on the seeds of the silk cotton.' It is probable that on account of the timidity of the bug, special methods of experimentation in the laboratory must be devised.

Success, however, attended an experiment which was carried out in England. Two bugs were placed in the toe of a black sock, which was then drawn half on to the foot. After some minutes a bite was felt on the dorsum of the foot, but in the attempt carefully to remove the sock the bug was disturbed and ceased biting; the reaction was an itching sensation with subsequent local swelling, and for a few days a red circular area on the dorsum of the foot was observed. Later the whole leg became swollen and oedematous, and intense itching occurred; there was no pain, however, and no enlargement of glands and no temperature. Parasites were not found in the blood, but there was eosinophilia reaching 50 per cent. In three weeks the swelling began to go down, and in a month was gone.

The bionomics of Dysdercus. This genus of bugs is best known from its association with cotton in most parts of the world, and it contains the majority of insects classed as cotton stainers. Maxwell-Lefroy gives a detailed account of the morphology and bionomics of *D. cingulatus* which occurs in India, as well as notes on other species; Ballou (1906) enumerates a large number of species which are found in the West Indies, with observations on the various stages

of the life-history in different species. Egg-laying commences within a few days after copulation is over, the eggs being laid to the number of up to a hundred in various sites, on the ground, under leaves, in the open bolls; Peacock, in *D. superstitiosus* in captivity, found egg clusters from twenty to one hundred and twenty; the egg-stage lasts about a week, and the larva which emerges undergoes during its growth five ecdyses. The adult is distinguished from the larva in most cases, according to Butler (1923), by the acquisition of wings; increase of number of joints in tarsi, and sometimes in antennae; transference of openings of scent glands from dorsal to ventral side; full development of sexual organs. The time taken for growth from the time the egg is laid till the adult bug stage is reached is forty-nine to eighty-six days for *D. cingulatus*, in India.

Food Plants. The chief food plants of these bugs are the following:—The cotton *Gossypium* sp.; the silk cotton, *Eriodendron anfractuosum*; the Okra or Bhindi, *Hibiscus esculentus*; the musk mallow, *Hibiscus abelmoschus*, and other plants of the Malvaceae.

Other food. *Dysdercus superstitiosus* was observed by me at Freetown feeding in large clusters on the carcase of a frog. Mansfield-Aders (1919-20), in his account of insects injurious to economic crops in the Protectorate of Zanzibar, states that he has on many occasions seen *Dysdercus fasciatus* feeding with avidity on fresh mammalian carcases, skins, and skulls. Of *D. superstitiosus* in Zanzibar, he says that it is by no means a common species on cotton, but that the silk cotton is commonly attacked by it.

Lamborn (1914-15) says of Southern Nigeria, 'During the dry season the Pyrrhocorid bug, *Dysdercus superstitiosus*, F., was found in some numbers . . . . and at this time they appeared to be able to thrive on almost any food, whether of animal or vegetable origin, for eight or ten were noticed feeding on a dead and sun-dried lizard and a batch of young nymphs was found on sheeps' excreta.'

Peacock (1913-14) gives a coloured plate of the stages of *D. superstitiosus*. He records the observation which he made of a number of young stainers about three weeks old sucking a dead snail.

Seasonal occurrence. Lefroy gives the following account of the

sequence of breeding and feeding habits of *D. cingulatus* at Pusa :—

April-May—Extensive breeding on Simul (silk cotton).
June-July—Feeding miscellaneous Bhindi, Hibiscus, etc.
August-November—Breeding in cotton.

'In most parts of India breeding is of necessity confined either to the cotton season, to the season when Bhindi is in pod, or to the season when the Simul is in bearing.' These observations are of interest with regard to the effect of season and the presence of particular food plants on the numbers and vitality of a single species.

Distribution of Species. Of equal interest are the observations made by Ballou on the distribution of different species in the West Indies. One species may extend for a distance and then, apparently without any change of environment, stop and give place to a different species. This sudden demarcation of the limits of a species is most noticeable in the case of *D. ruficollis*, and it is stated 'in many instances only one locality is known for each species, and most of the others occur only in a few adjoining countries or islands.' The distributions of *D. andreae* and *D. delauneyi* give good examples of localization. It is noted by Lefroy that *D. evanescens*, Dist., is recorded from Sikkim, the Khasi and Garo hills, Burma, from the Bor Ghat, Bombay, also from Chapra.

Modification of feeding habits. Butler (1923) considers the possibility of rapid change of food habit among bugs. He refers to two species of Capsidae which are even now gradually establishing, or indeed have established, themselves in orchards, viz., *Plesiocoris rugicollis* and *Orthotylus marginalis*; the natural food plants of these insects are various species of *Salix*, 'and the attack upon orchards indicates a startling change of taste brought about by the temptation of well-nurtured plantations of apple trees in their neighbourhood.' In captivity Ballou fed stainers on cotton seed, portions of unripe cotton bolls, bits of sugar cane and pieces of banana.

Duration of Life. In the insectary *D. cingulatus* was kept by Lefroy for four months; life was long when conditions were not favourable, *i.e.*, little food. I may observe here that a few specimens of *D. superstitiosus*, last larval instar and adult, which received no

food and which were kept at ordinary temperature, survived the voyage from Sierra Leone to England and lived for a week after arrival.

Bacterial parasites of Dysdercus spp. De Charmoy (1921) says that in Mauritius *Dysdercus* is known to transmit several bacterial diseases, and that it is the vector of an internal disease of the bolls similar to that described by Nowell and others in the West Indies.

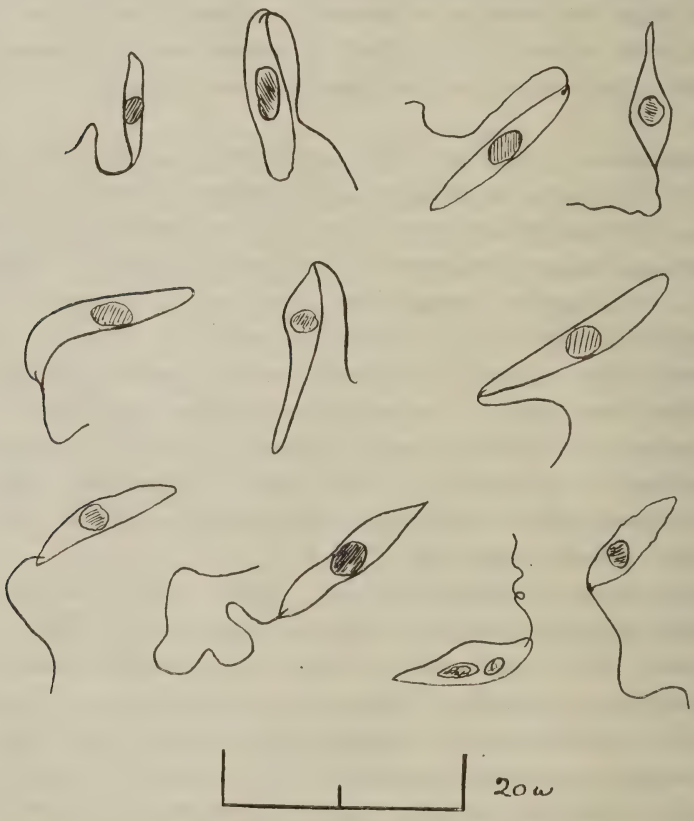

Fig. 1. Flagellate parasite of *Dysdercus superstitiosus*, F.

Flagellate parasite of Dysdercus superstitiosus, F. The bugs were found by me to be infected with a herpetomonas; it was present in various portions of the alimentary canal, and was recovered once from the coelomic fluid by cutting off the antenna near its base and examining the fluid which exuded; it was not found in the salivary glands, but as will be seen from the table subjoined, the number of dissections was limited:—

Flagellates (Herpetomonas) present in *Dysdercus superstitiosus* F.

Dissected	Infected	Rectum	Hind gut	Mid gut	Salivary glands	Coelomic Fluid
14	9	4	7	2	0	1

The occurrence of flagellates in Hemiptera-Heteroptera is well known. Patton and Cragg (1913) refer to the fact that tea, coffee and garden produce of all kinds are attacked by various species of bugs. They mention the family Lygaeidae, of which several species are infected with flagellates; *Oxycarenus laetus*, which is common on the cotton plant in Madras, is nearly always infected with a species of Herpetomonas; *Lygaeus pandarus* (*militaris*), which is common on the milk plant, *Calotropis gigantea*, is also infected with a flagellate of the same kind, *Herpetomonas lygaei*, which very closely resembles the parasite of Kala azar; *Lygaeus hospes* is infected with the same parasite. Of *H. lygaei*, Patton, the authors say that it is indistinguishable in its pre- and post-flagellate stages from the parasite of Kala azar as seen in man. The observations made by me on the flagellates of *Dysdercus superstitiosus* bear out these statements as regards the appearance of the Herpetomonas found, in the flagellate stage.

It is of interest to recall the discovery by Lafont (1909) of *Herpetomonas davidi* in the latex of *Euphorbia pilulifera* and to the presence of this flagellate in a species of Nysius. Miss Robertson has recorded a herpetomonas from the alimentary tract of *Dysdercus casiatus*, the red cotton bug of Uganda; only a few infected specimens were examined. A still more important observation of Miss Robertson is in connection with the species *Leptoglossus membranaceus*, of the family Coreidae in Uganda; she found in its alimentary tract a herpetomonas; the parasite was found very frequently to invade the salivary glands. Miss Robertson considers notable 'the independent development in a sucking insect of all the factors requisite for the transmission of a flagellate, parasitic in the intestine by way of the mouth-parts of the insect host.'

The significance of the observation which I have made on the biting capability of *Dysdercus superstitiosus*, F., is evident from a

consideration of the foregoing facts. The work of Laveran and Franchini (1913) and of Fantham and Porter (1915) went to prove that insect herpetomonas may, when injected into animals, produce effects analogous to those occurring in Kala azar, the flagellates resembling cultural forms of *Leishmania donovani*, giving rise to the non-flagellated rounded forms. The partial development in the bed bug obtained by Patton of Kala azar parasites by feeding experiments is important, and especially so in view of the non-success which has hitherto attended all attempts to find an insect vector of the parasite of this disease.

Roubaud and Franchini (1922) obtained in mice infection with Leishmania forms of parasite by allowing fleas having a natural infection to breed in their box. The infection, which proves fatal to the mice, was conveyed to fresh mice by means of subcutaneous injection of ground-up tissues. These workers also obtained a similar result in mice by injecting into them the faeces of fleas.

Although there is, as yet, no evidence that *Dysdercus superstitiosus*, F., is capable of removing blood from man, there is ample evidence that in biting it is capable of injecting an irritating substance under the skin. This irritating substance can on analogy be none other than the salivary fluid; it is clear, therefore, that all the conditions for transference of a parasite to man are provided if salivary infection is present. Dr. P. A. Maplestone reports that he has found infection of the salivary glands.

There is a hypothesis put forward by Stephens (1915) to explain the lack of success of infecting arthropods from Kala azar cases. On this hypothesis—the hemi-cyclic hypothesis—it is possible that a biting arthropod may infect man by its bite; the parasite injected into man grows and multiplies in the tissues but does not enter the peripheral blood in sufficient numbers to cause an infection in the alimentary tract of a fresh arthropod when biting; this is tantamount to saying that the parasite which gets into the arthropod from some other source than man reaches in man a *cul de sac* from which it cannot escape. The hemi-cyclic hypothesis, however capable it might be of explaining transmission of disease by the bites of insects which were yet not capable of sucking blood, need hardly be considered here, as we do not know what are the actual capabilities of such bugs in general in this respect.

It may be noted that whereas Kala azar has a very limited distribution, the bed bug, in which early development has been observed, is world wide in its range. If an insect transmitter of Kala azar is to be found, it is probable that it will be more restricted in its distribution than is Cimex. Their localized distribution, their seasonal dependence on certain forms of plant food and their evident adaptability, point to bugs of the Pyrrhocoridae and similar families as objects of study. I believe my observations and experiments indicate the necessity for an exhaustive investigation of all such forms, not only in countries where Kala azar abounds but also in countries in which Tropical Sore occurs.

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TYPHUS FEVER IN GREEK REFUGEES

BY

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During the last six months my duties in Greece have brought me into contact with about one thousand two hundred cases of typhus fever. From the nature of my work I saw the disease mainly from the standpoints of the medical administrator and the sanitary officer endeavouring to stamp it out in the areas allotted to the British Red Cross Society.

Epidemiology. The Greek refugees primarily brought the disease with them from Asia Minor and the Near East. With us the epidemic began at the end of January, 1923, gradually rose in number and severity of attacks, reached a maximum about the first fortnight in March, remained stationary a month or so and is now (June 22nd) dying out.

It is noteworthy that in the areas in which the refugees have been widely dispersed on the land there has been no typhus, or it has not gained a foothold when introduced. The brunt of the epidemic has been borne by the larger and medium-sized towns, the smaller either escaping, or being only slightly invaded. The city of Athens and its environs, Salonica, Patras, Corfu, and many other large towns, have suffered severely from the disease, whereas whole rural districts in Macedonia and Western Thrace have not been affected at all.

The housing conditions of the refugees, the hardships they have experienced, especially the insufficient food and exposure, have considerably reduced their stamina and vitality, and lowered their resistance to disease.

The outbreak attained its maximum of intensity in the mid-winter. In the colder weather the people are more crowded together, they wear thicker clothes which foster lice, and they bathe less frequently. Throughout the winter the refugees were packed with the ordinary civil population in the waiting-rooms of railway stations

and in other places; contact causes were therefore more frequent. In some small towns the number of refugees alone is equal to the former population; in others the refugees out-number the permanent inhabitants by two, or even three, to one. In the Landgada Valley (near Salonica) overcrowding has reached its possible limits. The ordinary population of the village of Landgada is five hundred; during the last six months one thousand six hundred refugees have been added to it. In one small refugee room, 9 ft. by 13 ft. as regards floor space and 5 ft. 6 ins. to the roof, I found four adult women and four children from 7 to 13 years old living; all the husbands were prisoners with the Turks; this was one of many similar overcrowded rooms above cowsheds. One could multiply instances of this sort; but overcrowding is only one of the many economic factors that enter into the epidemiology of typhus.

What has been happening is that infected refugees are necessarily brought into intimate contact with their non-infected comrades, all of whom were at one time (and to some extent are still) lice ridden. Infected lice are transferred to uninfected refugees. In the overcrowding that has arisen in nearly all the larger towns of Southern Greece, lousy infected refugees have also been brought into contact with the people of the permanent community, and the disease has thus spread to them. On several occasions I have seen, in organised refugee camps, typhus fever cases living with the uninfected and sharing the common bed, which is practically always the floor of the room or block they inhabit. The same takes place in railway stations where verminous typhus refugees infect the ordinary passengers in the waiting-rooms. Other hardships exist. A very large percentage of the refugees get only a bare subsistance allowance of food. There are districts in which the men who can work get no food dole and the out-of-work women only about six to eight ounces of bread or flour a day. For some months few refugees had a change of clothing; undergarments were especially scarce. With only one suit of clothes the practical difficulties of delousing are obvious; it is put through the steam disinfector and dried while the refugee is having his bath. It is necessary to emphasise that the sanitary condition under which the refugees lived for months after their coming to Greece was very bad; it has been greatly ameliorated.

Mode of Transmission. The transmission of typhus by body and clothes lice may be accepted as proved; the infected human louse is the intermediary in the transmission of typhus from typhus cases to the healthy—ordinarily there is no direct communication of the disease from man to man. If it were possible to exterminate human lice in an infected area, the disease would cease. Further, if we could rid all typhus cases of lice the disease would come to an end. All our radical preventive measures are based on these facts. Somehow it has got abroad that it is only the body-louse that acts as a carrier. This is not the case; the exculpation of *P. capitis* is a dangerous and pernicious theory to inculcate.

Period of Incubation. A generation or so ago, twelve days used to be given as the period of incubation. All recent experience of the disease in Russia and Greece shows this to be correct. It has been proved by the inoculation of human blood experimentally; inoculation of monkeys with infective human blood has likewise demonstrated it.

Most cases are admitted into infectious diseases hospitals on the fifth day of the disease, the next most frequent is the sixth day. This late admission arises from several causes—antipathy of the refugees to typhus hospitals, their ignorance as regards the nature of the disease, inability to obtain medical advice, overwork of the doctors, etc. In all but a very small proportion of cases the eruption is out by the time the patients reach the hospital.

Symptoms. In the vast majority of cases there are prodromata in the form of ill-defined malaise with vague symptoms for two or three or even four days before more severe indications arise. The real onset is well-defined. In typical cases the patient knows the day, often the hour, when he first felt genuinely ill and had to go to bed. The face is then somewhat flushed, the conjunctiva injected, the expression excited or dull, the tongue is coated, the lips and mouth dryer than normal; thirst, constipation, severe headache, pain in the back and limbs are complained of. Constipation is present in the great majority of cases, and often persists throughout the illness. By the time the patient is brought to hospital (fifth or sixth day) there is as a rule no doubt about the diagnosis. By the fifth day the mucous membranes are often implicated in the rash. The tongue has a well-marked white coat and is tending to become dry;

later the tongue is fissured, the mouth becomes offensive, sordes collect on the teeth, although these latter conditions can in many cases be prevented by proper nursing. Diarrhoea is not common in the early stage; about 20 per cent. of the cases develop it in the later stages. Asthenia and muscular debility are always present; patients can scarcely move in bed, they are often unable to protrude the tongue, and lose all expression. Emaciation in some cases towards the end of the disease is marked. In a small proportion of cases there is a definite crisis; in most cases, however, there is lysis, but a mixture of these beginning with lysis and ending in a crisis or the reverse may take place. The most common symptom of the real onset is headache, usually frontal, but sometimes mainly occipital. Conjunctival injection is present in four-fifths of the cases; it increases with the development of the eruption until lysis begins. Vomiting is present in about 25 per cent. of the cases. Sometimes it is severe and persisting for several days.

In many cases there is a distinctly reddish blush along the edge of the soft palate and pillars of the fauces, less frequently also slight congestion of the throat.

The Temperature. Whilst I believe it is possible to construct and describe what might be considered a normal temperature chart for an average case of moderately severe typhus, it is seldom that such a chart is met with in the wards in the natural course of the disease. A continuous or slightly remittent temperature of 103° or 103·5° F., during the first half of the second week, and then a slight daily decline until the eleventh or twelfth day, when there is a more decided remission with abatement of all the symptoms, is ordinarily what may be expected. Then there is another rise, say on the twelfth day, and a remission, and a second similar oscillation though less marked, with a decline to normal, and even a third, the whole lysis occupying forty-eight to sixty or seventy-two hours. Irregular temperatures are also met with. Again a definite crisis with a fall of temperature to normal in twenty-four or thirty-six hours may occur, although this is exceptional. After the temperature has dropped and the symptoms have disappeared, the drop may be to sub-normal for some days.

Of two hundred and forty-six cases that recovered, in nineteen the temperature was normal on the twelfth day, thirty-seven on the

thirteenth day, sixty-six on the fourteenth day, forty on the fifteenth day, and twenty-two on the sixteenth day.

The Pulse. Normally in ordinary cases, the pulse-curve follows that of the temperature. The pulse, however, is liable to show much variation; sometimes marked oscillations occur in the twenty-four hours, being at one time ninety and at another one hundred and twenty to one hundred and thirty in a minute. Dicrotism is not uncommon, especially towards the end of the second week. With the tendency to cyanosis so commonly seen in the late stage of the second week, the pulse is often absent at the wrist. The state of the patient's lungs appear to me greatly to affect the pulse, especially in wide-spread broncho-pneumonia. The pulse is markedly improved on the first signs of defervescence; in a few a very slow pulse is present in convalescence. The respiration curve varies less than that of the pulse.

The Respirations. In uncomplicated cases with moderate temperatures, the respirations are shallow and from thirty to thirty-five per minute, they vary little and without real dyspnoea at any stage. In similar cases with broncho-pneumonia, dyspnoea becomes a serious symptom.

The Eruption. This first appears on the evening of the fourth day in the form of discrete and well-defined pink or roseolar spots which may be round, oval or irregular, varying from 2 to 5 mm. in diameter, vanishing on pressure; they are seldom palpable at this stage, but they are widespread though scanty, and are seen on the abdomen, back, chest, shoulders, arms, legs and feet; they are rare on the face and head. In this early stage the rash described is not very obvious, it may require careful scrutiny to find it. The macules then become larger and of a bright red colour, next assuming a purplish-red hue running into dark purple. At this stage the tendency is for the eruption not to disappear on pressure, but this is not invariable—in many cases ending fatally with a deep coloured eruption before death, no sign of it remains *post-mortem.* When the eruption is fully developed on the eighth or ninth day, well-defined dark coloured patechial areas which do not disappear on pressure are seen, besides less-defined patches of much lighter colour which do not disappear on pressure. In all severe cases with typical eruption, these erythematous and patechial patches are met with

during the second week of the disease. In blonde boys and girls, during the early stage of the disease, we sometimes see on the chest, neck, arms, and occasionally on the abdomen, an irregular or blotchy erythema which vanishes before the real eruption is developed. In the second week the eruption has a multiform character—roseolar patches, red spots, maculae, small patechiae and large plaques typically patechial are seen; this multiformity is well seen on the shoulders and back, lower part of the abdomen and hips, outer surface of the arms and forearms, on which places what has been admirably named 'subcuticular mottling' is also visible. The eruption may, however, vary from consisting of only faint roseolar slightly raised spots to large ecchymotic looking patches. By the end of the second week little of the eruption is left. In some cases the general lousiness antecedent to the onset of the disease leads to considerable skin irritation with scratching and local secondary infections, which may initially be rather puzzling. Chronic pediculosis and pityriasis versicolor (both common in refugees) are the chief conditions of the skin likely to lead to confusion in a diagnosis based on the eruption alone. In about 1 per cent. of typhus cases there is either no eruption or only a faint roseolar one; this is more frequently the case in children and adolescents; in these cases the Wiel-Felix reaction is present. It is useful to carry about a good hand-lens, and, to bring out the eruption, rub into the skin some petrolatum; the lesions are then seen to consist of a congeries of dark red blood vessels.

Insomnia is one of the commonest symptoms; the majority of cases suffer from it during the first week of the disease.

In about 25 per cent. of the cases some form of mental disturbance is present on admission, and on the seventh or eighth day delirium. In cases that are running a fatal course, the delirium often passes into coma more or less complete. Cough is one of the most constant symptoms. In the earlier stage it is short and dry. Later on the expectoration may become profuse and mucopurulent. In a number of cases patches of lobular pneumonia occur. This is a common terminal condition in fatal cases. Diarrhoea is common in the later stages of the disease, and is then sometimes associated with rectal incontinence. Parotitis is one of the more serious complications; I saw altogether twenty of these cases, and as many as three in a

ward of thirty patients. Otitis media occurs in a small percentage of cases; it may become chronic. Deafness is a marked feature in many cases of typhus, during the late stage of the disease; this is quite distinct from the dullness of intellect that exists during that stage.

The spleen can be felt in about two-fifths of the cases; sometimes it is of considerable size. I do not lay stress on this, as many patients have a history of old malarial infection.

In all hospitals dozens of recurrent fever were sent in as typhus. In relapsing fever the *sudden* onset with rapid rise of temperature, severe headache, pains in the back and extremities, absence of dulness and apathy and (ordinarily) of rash, the presence of a moist tongue and of *S. obermeieri* in the blood, and later in the disease, more or less anaemia, should be sufficiently distinctive. In the Salonica Hospital the records show that eight cases of typhus and relapsing fever ran their course concurrently in the same persons.

The use of neo-salvarsan for the recurrent fever did not affect the normal progress of the typhus. The combined infection seems to suggest that the same louse may be able to carry the virus of typhus and *S. obermeieri* and inoculate them at the same time. Of course, two or more lice, each with a single infection, may have attacked these cases. It seems to be established that, contrary to the rule with intermediate hosts, the virus of typhus eventually kills the louse. In many, typhus and influenza ran together, the latter disease being also epidemic at the time. Hundreds of cases of both smallpox and typhus have been admitted into infectious diseases hospitals; in no single instance have both diseases been met with in the same patient at the same time; in one, typhus followed smallpox from infection acquired in the hospital.

Wiel-Felix Reaction. In typhus this reaction is very distinctive. It owes its origin to the discovery of the fact that what are called the 'X' strains of *B. proteus* are agglutinated by the serum of typhus cases. The special strains that do this are X2 and X19; these were primarily obtained from typhus urine. The macroscopic method is here usually adopted. The minimum dilution accepted as positive is 1 to 100. Practically the serum of every typhus fever case after the eighth day is positive, whilst that of other fevers is negative.

Complications. Bronchitis of greater or lesser severity is present in a large proportion of cases. Broncho-pneumonia is another common complication affecting the bases of both lungs. Pleurisy is much less frequent. Other complications are—severe diarrhoea, myocarditis, cardiac dilatation, parotitis, otitis media, conjunctivitis, keratitis, gangrene of the toes, bedsores, etc.

Prophylaxis. Medical men, nurses, and all sick attendants looking after typhus cases should be thoroughly protected from lice by suitable white cotton or linen clothing from head to foot before commencing their work, and have a bath and complete change of clothing after finishing their day's work. Even with these precautions infections will occur, but without them infection is all but certain, sooner or later, in those not immunised artificially, or by a previous attack of the disease.

Etiology. It would appear that the micro-organism of typhus passes through a development stage in the louse; in that insect it is intracellular, develops, is set free and is introduced into man. In man it is said to become intracellular once more, and from the infected cell to be thrown into the blood with its toxins. The members of the Typhus Fever Commission of the League of the Red Cross Societies to Poland, however, found no evidence of the development of the micro-organism in the louse, but they arrived at certain important conclusions as the result of their work.

Summarised, their conclusions are:—

Pathology. The lesions of typhus appear to be situated in the blood vessels of the skin, central nervous system, skeletal muscles, and to a lesser extent in some of the viscera—heart, kidney and testes. Typhus is considered to be a disease of the smaller blood vessels, and localises almost exclusively in the vascular endothelium. The reaction to the parasite is shown primarily by degenerative changes giving rise to thrombi in the blood-vessels, and by a proliferative reaction on the part of the endothelium and neuroglia which give rise to the characteristic 'nodules' of the disease in the skin and central nervous system. When lice are fed on typhus cases, while they develop *Rickettsia prawazeki* with great regularity they develop no other form of micro-organism. All lice so far do not become infective, but why this is so is not determined. Infection with *R. prawazeki* eventually kills the louse, which is an exceptional

effect of a parasite upon its intermediate host. *R. prawazeki* escapes from the alimentary tract with the faeces, and therefore may be introduced by scratching or by the mouth-parts of the louse becoming soiled with the faeces. *Rickettsia* has not been found in the salivary glands or in the mouth-parts of the louse.

Mortality. The average mortality in the infectious diseases hospitals near Athens is roughly 10 per cent.; it is, however, higher in some towns, such as Corfu, Patras, Volo, etc. It varies also greatly at different ages. In refugees of 50 years and over, the death rate is high, reaching in some towns 50 per cent.

It is necessary that some definite routine plan should be adopted in admitting typhus fever cases into hospital and distributing them in wards. The first requirement is a receiving-room, to which all patients are primarily brought. Here the hair of the head is rapidly cut off with a machine clipper, the hair of the axillae and pubes being shaved off; the hair is to be burnt. The receiving-room should communicate with the room or other area containing the steam-disinfector on the one hand, and with the bathroom on the other; this latter should lead to the dressing-room. After removal of the hair the patient is put on a stretcher and conveyed to the room containing the disinfector. Here he discards everything that he brings with him, which is disinfected. He is taken to the bathroom and bathed. He is then put on a clean stretcher and removed to the dressing-room; here he receives a suit of clean hospital clothing and is taken to the ward he is to occupy. There must be no remission in this routine; it must be thoroughly carried out if the wards are to be kept free from infected lice. Nothing that the patient brings with him to hospital should enter the ward.

Thoroughly deloused typhus cases are perfectly innocuous to the uninfected, and if we are quite confident as regards the efficiency of our delousing arrangements there is no reason for putting them in different wards.

Treatment. Typhus patients should be kept in bed throughout the pyrexial stage of the disease, and for a fortnight after the fever has subsided. Constipation is best relieved by a simple enema every second day. In some cases the catheter has to be used to drain off the urine. In the early stage, when sleeplessness, irritability, general discomfort and delirium are present, small doses of morphine

give satisfactory results. The morphine may later on be replaced by veronal, sulphonal, paraldehyde or chloral hydrate, if one or other is called for in mild delirium or insomnia. For prolonged and marked active delirium, hyoscine, hypodermically, is a valuable drug. The most popular stimulant is hypodermic injections of camphor (5 grains in 1 c.c. of olive oil or ether put up in ampoules); strychnine is also largely employed.

Prophylactic Inoculations. As a substitute for a prophylactic vaccine the blood of typhus fever cases has been inoculated, and the virus thus introduced in a living state. In using the living virus, Kusama injected monkeys with a fairly definite minimum dose of typhus blood, known as the minimal morbid dose, to bring about an attack. If a smaller dose is given no attack occurs, but a state of immunity is produced and the animal can tolerate many times the minimal morbid dose without ill-effects. This active form has so far not been used; the killed virus is the one that is used here. Whether it has any prophylactic value is uncertain.

Prevention. The amount of actual physical labour connected with the preventive work associated with typhus may be understood by describing what took place in two large blocks housing nearly two thousand refugees, in March last. From the 12th to the 17th, the whole camp was deloused and all bedding and clothing put through the steam disinfectors. The entire floors were washed with disinfectants and the walls whitewashed. This meant that every room had to be emptied of its entire contents while this was going on; then all the belongings of the refugees returned to the cleaned rooms, which were shut off from those awaiting their turn. It likewise meant giving a complete hot bath to everyone in the camp under supervision. From the 19th to the 24th there was a renewal of the bathing. A slight recrudescence of typhus after this necessitated a repetition of the processes carried out previously. This was done from the 26th to the 31st March. All men's and boys' heads were shaved, and all girls up to the age of fourteen had their hair bobbed. All cases of actual typhus, of course, had their heads shaved. Our nursing sisters also used on the heads of the refugees in camp the mixture of equal parts of kerosene and olive oil we employ to free nits from hair, and they distributed N.C.1 powder (naphthaline ninety-six parts, creosote two parts, iodoform two parts)

which was in little bags to be worn for a week, when a fresh bag is issued. The idea is that the heat of the body helps to vaporize the ingredients, and in this way is created a louse-destroying atmosphere next the skin and the clothes.

By order of the Government, refugees are allowed to leave the camps to work or to search for work, so long as their identity cards show that they are free from infectivity, which means free from lice. Many of the people allowed outside run the risk of acquiring the disease in other refugee camps which they visit, or elsewhere. This at present is unavoidable. Another weak link in the chain of preventive measures was the fact that for months after their arrival in Greece, a large percentage of the refugees did not possess a change of underclothes. It is obvious that where the people have only one suit of clothing there must be grave difficulties in rendering them lice free. An adequate supply of steam disinfectors for delousing clothes and bedding is almost indispensable; in their absence the task is most laborious. In small communities, Serbian barrels and other such improvisations may be useful, but in dealing with large masses of people they are futile.

I believe it to be well worth while in every camp to endeavour to educate the people in regard to the nature of typhus and the principles that underlie its eradication and prevention. We placarded leaflets in this connection, and also had them read out periodically for the benefit of the illiterate.

The specific measures now indicated appear to be to disperse the refugees from overcrowded towns to rural areas as much as possible. Another urgent requirement in many towns is an increased supply of water, which should be available to every house, or at least be within easy reach. In some camps the water-supply is decidedly defective, which interferes with the bathing arrangements, steam-disinfection of clothing and bedding and ordinary laundry-work—serious obstacles when endeavouring to eradicate typhus fever from an infected camp. The Greek Administration is endeavouring in all possible ways to remove this difficulty.

Every effort to quarantine contacts has failed, and all things considered it is not reasonable to expect it. All we want is that the person who is a contact should be watched definitely for twenty-one days, that is the time we laid down as the incubating

period. We endeavoured, as far as was possible, to render these certificated people free from lice; we felt that if thus free they could not communicate typhus, even if they acquired it themselves in the meantime. The quarantining of contacts in a small country which contains over a million refugees, many thousands of whom are contacts and are either in search of work or have daily to go to work from their camps and return to them, is an impossibility. It may be easy to do this in a limited area of infection with a stationary and disciplined population; it is an impossibility among refugees. The hope is that nature will step in during the summer and bring the epidemic to an end by stopping the multiplication of lice, by unfavourable meteorological conditions, and that by the beginning of the next typhus season the economic and other conditions will have altered for the better. At the present moment it is certain that the economic state of the refugees is decidedly antagonistic to effecting a cessation of the disease by the adoption of ordinary preventive measures.

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THE VALUE OF THE SACHS-GEORGI REACTION IN THE SEROLOGICAL DIAGNOSIS OF SYPHILIS

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In order to determine to what extent the Sachs-Georgi could be relied upon to replace the Wassermann reaction in this country (Palestine), I commenced to perform a parallel series of tests in the latter part of 1921. It was by no means difficult to secure suitable material, inasmuch as it was possible to collect the sera of a large number of untreated cases which gave true clinical manifestations of syphilis in one or other of its various stages and forms. It was not so easy, however, to gather together sera from cases undergoing treatment, nor to follow up the effect of such treatment on the reactions.

Doubtless the disappearance of the then existing lesions led those affected into the belief that they were cured, and this deduction of ' out of sight, out of mind ' is strengthened by the fact that only comparatively few names appear on the register more than once.

The conclusions arrived at, both from the performance of the ordinary tests and from certain experiments carried out bearing on the subject, are of interest, if, in one or two particulars, somewhat puzzling.

I shall in this paper detail :—

(*a*) the methods employed—including the technique of the Sachs-Georgi reaction as carried out during the whole series; (which technique I have found by experience easiest and most suitable);

(*b*) the actual results of the two reactions;

(*c*) the relative percentage of agreement;

(*d*) certain fallacies of the Sachs-Georgi reaction—certain experiments made with a view to explain the cause of several, and certain theoretical observations; and finally,

(*e*) the conclusions and inferences drawn.

(*A*) METHODS EMPLOYED

For the performance of the Wassermann reaction, the Boas modification of the original has always been employed; the results of this technique have been so uniformly dependable that no other method is permitted in these laboratories. The antigen and haemolytic serum are both prepared by Burroughs, Wellcome & Co., and are forwarded every three months.

The technique of the Sachs-Georgi reaction is simplicity itself, and will be briefly outlined here.

(*a*) *Antigen.* This is supplied quarterly by Burroughs, Wellcome & Co., and although, in 1921, I experimented with various antigens, I found that prepared by the above firm so reliable that it was adopted in preference to the others.

A dilution of 1 in 20 is required.

1 c.c. antigen is pipetted into the ordinary 1 inch by 6 inches test-tube. To this is added 1 c.c. normal saline freshly prepared—the saline being allowed to run slowly down the side of the test-tube held in the sloping position.

The tube is agitated gently during the process of admixture. 18 c.c. normal saline are now to be added. The test-tube is held vertical and a pipette containing the 18 c.c. held with its point midway over the upper end of the tube. By gentle pressure on the indiarubber teat, the saline is allowed to fall drop by drop the height of the test-tube into the mixture; during the whole time this is being effected, the tube is gently shaken from side to side. The tube now containing 20 c.c. of 1 in 20 dilution is inverted slowly against the palm of the hand several times. The resultant antigen, ready for use, is a shimmering, somewhat opaque fluid with just a milky tint, giving the appearance of watered silk.

(*b*) *Patient's serum.* The serum is inactivated as in the Wassermann reaction, for half an hour at 56° C. When sera have to be sent to the laboratory from a distance, the medical officers are issued with instructions that blood must be taken with all aseptic precautions, and that the serum should be allowed to separate out in a sterile test-tube (preferably kept over night in the sloping position) and transferred to sterile bottles which are then carefully stoppered and sealed. In this way not only is haemolysis prevented

but also is obviated one of the chief causes of failure (to be detailed below) of the Sachs-Georgi reaction and discrepancy between this reaction and the Wassermann.

(*c*) *Method of setting up the reaction.* Four Wassermann tubes are used for each case, and dilutions of patient's serum made, 1 in 5, 1 in 10, 1 in 20, and 1 in 40, with normal saline freshly prepared.

Into tube 1 is placed 1·6 c.c. and into tubes 2, 3, and 4, 1 c.c. of normal saline.

To tube 1 is added 0·4 c.c. patient's serum and the contents of the tube are thoroughly mixed with a pipette. One-half the contents of tube 1 is transferred to tube 2 and thorough mixture made again.

To tube 3, one-half the contents of tube 2 is added, and again, after mixture, one-half the contents of tube 3 is transferred to tube 4. After suitable mixing, one-half of the tube 4 contents is now discarded.

Tube 1 now contains 1 c.c. of 1 in 5 dilution of patient's serum.
Tube 2 ,, ,, 1 c.c. of 1 in 10 ,, ,, ,,
Tube 3 ,, ,, 1 c.c. of 1 in 20 ,, ,, ,,
Tube 4 ,, ,, 1 c.c. of 1 in 40 ,, ,, ,,

To tubes 1, 2, 3, and 4 is added 0·5 c.c. of the already prepared 1 in 20 dilution of antigen, and the contents of all tubes are thoroughly mixed by inverting the tubes between the thumb and different fingers several times.

The tubes are now placed in an ordinary Wassermann bath and kept at a temperature of 37° C.

Mixing by inverting the tubes is done as a routine measure three times during the first twenty minutes, unless signs of a positive reaction have already made themselves manifest.

At the end of one hour, six hours, eighteen hours and twenty-four hours, the results are read and recorded. Saline negative and positive controls are set up in similar fashion according to the method described above. 1 c.c. of normal saline replaces the 1 c.c. patient's serum in the saline control, while 0·4 c.c. of known negative and positive sera will be added respectively to the 1·6 c.c. normal saline in the first tubes of the series instead of 0·4 c.c. of patient's serum.

For the positive control is taken one-half of a serum previously

proved to be positive both by the Wassermann and Sachs-Georgi tests.

Reading of Results.

In well marked cases there is no difficulty, even for the most inexperienced, in reading both positive and negative reactions. The negative tubes show at the end of eighteen to twenty-four hours the same uniform shimmering fluid which on agitation presents the appearance of watered silk. The impression of the term 'watered silk' must be very carefully appreciated by the beginner, as upon that impression depends future success or failure in reading results.

The use of a hand-lens in doubtful cases is advocated and has proved of service to my assistants, but personally I have found the readings of 'granular positives' relatively simple, as my own visual acuity is − 5 D.

Positive results, if very marked, give the appearance of snow-flakes suspended throughout a clear fluid, and from the gradual settling of these white masses, a snowy layer ultimately forms at the bottom of the test-tube.

The supernatent fluid is then absolutely clear. The flocculations in this class are termed massive, and the precipitate heavy; we register it as XXX.

If defintely positive but less marked, the tubes give the appearance met with in ordinary bacterial agglutinations when a high titre serum is used.

The flocculi tend to deposit later and leave a clear supernatent fluid; this we regard as XX.

In the third group considerable difficulty may be experienced in differentiating the finely granular flocculi of a positive from the homogeneous suspension of the antigen. The appearance presented is somewhat like particles of dust shewn up by a ray of bright sunlight suddenly penetrating a dark room. There is little or no tendency for these particles to deposit, and the fluid does not become clear as in positives described above, this we record as X.

If doubt still exists, recourse may be made to having the tubes centrifuged to see whether a deposit can be obtained.

It has not been considered necessary to adopt any artificial lighting device to help in the reading of the results.

In passing, it may be remarked that on numerous occasions tubes

have shown what appeared to be positive reactions within the first few hours, but at the end of eighteen to twenty-four hours this appearance had completely disappeared; and also, it has been noted that gentle agitation may produce such a disappearance in what may be called pseudo-positives.

I shall have occasion later to refer to the occurrence of positive readings in the Sachs-Georgi reaction—actual positives which do not disappear as those mentioned in the last paragraph, but which owe their existence, although not to syphilis, to certain definite causes.

(*B*) ACTUAL RESULTS

1. *Complete* POSITIVE *agreement was obtained between the Wassermann and Sachs-Georgi reactions*:—

(*a*) In three hundred and ten *untreated* cases submitted from venereal clinics and hospitals with a definite history of syphilis.

Analysis of these three hundred and ten cases:—

(1) *Primary stage.* Forty cases.

(2) *Secondary stage.* Two hundred and fifty cases.

These cases without exception showed typical pictures of secondary syphilis-rash, sore throat, mucous patches, etc. They had neglected the primary stage completely, and had come to seek advice only when the rash, fever, and constitutional symptoms manifested themselves.

(3) *Tertiary stage.* Eight cases.

Table I.

Cases	History	Wassermann reaction	Sachs-Georgi reaction
4	Gummata	XXX	XX
1	General paralysis	XXX	XX
2	Cerebro-spinal fluids in cases showing nervous symptoms	XX	XX
1	Hemiplegia	XX	XX

(4) *Congenital syphilitics.* Five cases.

These were discovered during the routine examination of inmates of an orphanage.

(5) In seven cases where the patients had several abortions.

Table II.

Case	History	Wassermann reaction	Sachs-Georgi reaction
1	3 abortions; 2 still births	XXX	XXX
2	5 abortions	XXX	XX
3	6 abortions	XXX	XX
4	5 abortions	XXX	XX
5	2 abortions	XXX	XXX
6	'Several abortions'	XXX	XX
7	'Several abortions'	XXX	XX

In case No. 1, husband had syphilis five years ago, and was treated with full course of Neo-Salvarsan injections. Serum tested by both reactions on same day as patient gave completely negative readings.

(*b*) In certain *treated* cases.

Eight cases showed markedly positive reactions. Of these cases one had received two injections of Neo-Salvarsan, and four had had 'complete' treatment (see below).

(*c*) In ninety-seven cases where no history accompanied the specimens.

2. *Negative agreements.*

Negative readings in all tubes of both reactions were obtained in five hundred and fifty cases of sera submitted without definite history, and as part of routine examinations. In two cases previously reacting XXX to both tests, sera now are completely negative to both.

3. *Partial agreements.*

These may be best illustrated as follows:—

Table III.

Cases	Wassermann reaction	Sachs-Georgi reaction	Remarks
4	XXX	X	1 with 4 injections of Neo-Salvarsan.

4. *Non-agreement.*

(*a*) In forty-five cases submitted, mostly without history, the Wassermann reaction was definitely positive, the Sachs-Georgi negative. In thirteen cases, however, details were supplied-symptoms of primary stage 8, of secondary stage 5.

(*b*) In twenty-one cases the Sachs-Georgi reaction was positive, the Wassermann completely negative. This figure does not include positive Sachs-Georgi reactions obtained during experiments performed. These latter will be detailed below.

(*c*) In twenty-three treated cases, the Wassermann was positive, the Sachs-Georgi negative.

(These cases were either partially or completely treated according to the following routine method practised here.

The course of Neo-Salvarsan injections is:—

1st injection	...	0·45 grammes.
2nd injection	...	0·60 grammes one week later.
3rd injection	...	0·75 ,, ,, ,, ,,
4th injection	...	0·90 ,, ,, ,, ,,
5th injection	...	0·90 ,, ,, ,, ,,)

Table IV.

No. of cases	Wassermann reaction	Sachs-Georgi reaction	Remarks
14	XX	o	'partially' treated
2	XXX	o	'with 3 injections'
7	X	o	'complete treatment'

(C) THE RELATIVE PERCENTAGE OF AGREEMENT BETWEEN THE REACTIONS

The calculations are based upon one thousand and thirty-seven examinations of sera.

1. *Positive agreement.*

In all, four hundred and eighty-seven showed a well-marked positive Wassermann, while with the corresponding sera the Sachs-

Georgi reaction showed positive readings in four hundred and nineteen.

The positive agreement therefore is 86 per cent.

2. *Negative agreement.*

Whereas the Wassermann reaction was negative in five hundred and fifty cases, the Sachs-Georgi was negative in six hundred and eighteen.

The negative agreement is therefore 89 per cent.

(*D*) CERTAIN FALLACIES OF THE SACHS-GEORGI REACTION WITH CERTAIN EXPERIMENTS MADE WITH A VIEW TO EXPLAIN THE CAUSE OF SEVERAL, AND WITH CERTAIN THEORETICAL OBSERVATIONS

(1) The presence in the serum to be tested of contaminating organisms renders the findings of the Sachs-Georgi reaction of no value whatever.

The first disparities between the Wassermann and Sachs-Georgi reactions here occurred in cases where the sera were sent from a distance, and when two or three days elapsed between collection and examination.

In those cases, fortunately, little doubt could exist as to contamination, as the sera were turbid and malodorous on arrival. Actual proof was obtained by plating on culture medium. A new series of reactions was performed in the case of those sera proved contaminated: they were re-submitted, and after transmission, arrived in a sterile condition. On these occasions they showed a completely negative reading where previously they had shown a strongly positive reaction. I refer in this connection only to sera which reacted positively to the Sachs-Georgi when the Wassermann reaction remained negative.

Further proof was adduced by the following simple experiment:—

A normal blood was drawn off in the laboratory, and the serum proved negative by both reactions.

The serum was then artificially infected with (*a*) *B. typhosus* and (*b*) *B. subtilis*, and then after suitable incubation the sera were set up in the ordinary dilutions, with the following results:—

Table V.

Case		Wassermann reaction	Sachs-Georgi reaction	Remarks
1	Normal blood serum ...	o	o	—
2	The same serum infected with *B. subtilis*	o	XXX	In both cases flocculation massive, precipitation heavy.
3	The same serum infected with *B. typhosus*	o	XXX	

If, then, the reaction could be completely altered by the presence of contaminating organisms in the serum, I considered it advisable to test the sera of patients suffering from certain diseases, to determine whether positive results in the Sachs-Georgi might be obtained here also.

The results are of interest, and although unfortunately at present the number of such examinations is small, yet I hope later to submit the results of examinations of many sera collected from patients suffering from the diseases most common in Palestine, so as to determine to what extent the Sachs-Georgi reaction is influenced by such.

First I was able to obtain six sera from lepers in Jerusalem, and the particulars and reactions are as follows:—

Table VI.

Case		Treatment	Wassermann reaction	Sachs-Georgi reaction
1	Nodular leprosy ...	Injections with Ol. Chaulmoograe for 1½ years	o	o
2	Nodular leprosy ...	Injections with Ol. Chaulmoograe for 3 months	o	X
3	Nerve leprosy ...	Untreated	o	XX
4	Nerve leprosy ...	Untreated	o	o
5	Nodular leprosy (?)	Untreated	XXX	XXX
6	Nodular leprosy ...	Treated with Ol. Chaulmoograe ...	o	XXX

From these examinations, no conclusions can be made.

In addition, however, the following examinations were made all at the same time with the fullest possible controls. All precautions were taken to ensure (*a*) freedom of the sera from contamination, except where otherwise indicated; (*b*) that the reactions were made in the dilutions considered essential; (*c*) that the reaction of the normal saline used was PH7 ; (*d*) that the readings were made after one hour, six, eighteen, and twenty-four hours, and that pseudo-flocculation was eliminated.

As these examinations have been performed for experimental purposes, the results have not been included in the calculations made as regards positive and negative agreements.

Table VII.

No. of case	History	Wassermann reaction	Sachs-Georgi reaction	Remarks
1	Street case taken at random ...	o	o	
2	Negative control	o	o	
3	Positive control	XXX	XXX	
4	Hospital routine examination ...	o	o	
5	Routine examination from clinic ...	o	o	
6	Case of untreated syphilis (stage 2)	XXX	XXX	
7	Sore throat, rash, typical 2nd stage	XXX	o	Disparity 1
8	Aneurysm of Aorta	XXX	o	Disparity 2
9	Case of syphilis previously reacting strongly to both reactions, treated with 4 injections of Neo-Salvarsan	XXX	XXX	Treated according to table above.
10	Case of syphilis previously proved positive to both reactions—treated with 4 Neo-Salvarsan injections	XXX	XXX	
11	Sore throat, mucuous patches ...	XXX	XXX	
12	Soldier—contracted syphilis 1921—fully treated	o	o	
13	Soldier—Syphilis 1921—full course of treatment	o	o	
14	Soldier—primary case chancre ...	XXX	XXX	
15	Soldier—ulceration of palate ...	XXX	XXX	

TABLE VII—*Continued.*

No. of case	History	Wassermann reaction	Sachs-Georgi reaction	Remarks
16	Male—previous syphilis untreated	XXX	XXX	
17	Female—married case 16 last year; has had a child full term, showing signs of congenital syphilis ...	XXX	XXX	
18	Untreated secondary stage syphilis	XXX	XXX	
19	Arthritis (right ankle)	XXX	XX	
20	Syphilis—after full treatment ...	o	o	
21	Periostitis of femur	X	o	
22	Ulceration of buttock	o	o	Pseudo-flocculation in first tube only.
23	Routine examination—no history	o	o	
24	Routine examination—no history	o	o	
25	Routine examination—no history	o	o	
26	Routine examination—no history	o	o	
27	Pharyngitis	o	o	
28	Chronic Jaundice	o	o	
29	Tumour of R. hypochondrium independent of liver; inguinal glands enlarged; pigmentation of legs and ankles	o	XX	Disparity 3
30	General malaise	o	o	Pseudo-flocculation in 1 tube only.
31	Pustular eruption	o	o	Pseudo-flocculation in 1 tube only.
32	Enlarged spleen (malarial)	o	o	
33	No history	o	o	
34	No history	o	o	
35	General malaise	o	o	
36	Serum from case of Typhus fever showing all clinical symptoms and reacting to Weil-Felix test (*B. proteus* × 19) 1 in 400 ...	o	XXX	Disparity 4
37	Serum from case of Typhus fever Weil-Felix reaction 1 in 400 ...	o	XXX	Disparity 5
38	Serum from case of Typhus fever Weil-Felix reaction 1 in 200 ...	o	X	Disparity 6

TABLE VII—*Continued.*

No. of case	History	Wassermann reaction	Sachs-Georgi reaction	Remarks
39	Serum from case of Typhus fever Weil-Felix reaction 1 in 400 ...	XXX	o	Disparity 7
40	Serum from case of Relapsing fever	o	o	
41	Normal blood serum	o	o	
42	Serum of 41 infected with *B. typhosus*	o	XX	Disparity 8
43	Serum infected with *B. subtilis* ...	o	XX	Disparity 9
44	Serum of patient suffering from acute lobar pneumonia	o	XX	Disparity 10
45	Serum—another patient recovering from lobar pneumonia	o	XX	Disparity 11

In the case marked pseudo-flocculation in tube 1, it was found, as previously mentioned under 'reading of results,' that gentle shaking of the tubes in question produced an immediate disappearance of the seeming flocculi.

The importance of the two precautions—(*a*) always to shake the tubes gently before reading; (*b*) not to give definite reading until after the tubes have been set up for eighteen to twenty-four hours—cannot be too strongly emphasized.

I do not attempt to give any explanation of the disparities between the two reactions in the cases instanced above, but these few findings would seem to suggest that the presence of organisms, or products of organisms, in the patient's blood might well have some effect in reducing the value of the Sachs-Georgi unless the fullest history accompanies each serum submitted.

(2) Granted the complete sterility of the sera submitted, could any reason be advanced for the Sachs-Georgi reaction giving a strongly positive reading in the presence of a non-syphilitic serum?

From time to time in these laboratories the distilled water had been shown to be definitely acid, PH −, which acidity was due to various causes, principally carbon dioxide or absorption from an atmosphere containing acid fumes. Now it is a well-known fact that in agglutination tests, so-called 'pseudo-clumping' may occur on account of excessive acidity of the culture medium (see

Biggs & Park, American Journal of Medical Science, 1897; Block, B.M.J., 1897).

Agglutination of bacteria by acids in definite concentration can be carried out, and the phenomenon seems to depend upon the hydrogen ion concentration. In this connection the clumping of bacteria in acid agglutination is analogous to the clumping of colloidal suspensions of any kind, and the clumping or agglutination is merely a physical phenomenon, determined by the colloidal equilibrium of the bacteria in suspension. To digress a moment—this acid clumping was well exemplified by the experience of one of my assistants in Jaffa. He had been getting positive results in every Widal including controls performed in the laboratory there; and on enquiry being made it was discovered that the new laboratory attendant had rinsed the agglutination tubes after immersion in acid only perfunctorily. Reasoning that a similar or analogous phenomenon could occur in the performance of the Sachs-Georgi test, I had certain experiments carried out which seem to prove the likelihood of the supposition.

(*a*) Twelve sera were examined by the Wassermann and Sachs-Georgi tests. The diluent of antigen and serum was normal saline with a reaction of PH 7.

A third series was put up for the Sachs-Georgi test, but in this series the diluent of antigen and serum gave a reaction of PH 5.

The reading of the three series at the end of eighteen hours are as under:—

Table VIII.

No.	Wassermann reaction	Sachs-Georgi reaction (Normal saline used, PH. 7)	Sachs-Georgi reaction (Saline used, PH. 5)
1	XXX	XXX	XXX
2	XXX	XXX	XXX
3	o	o	XX
4	o	o	XX
5	o	o	o
6	o	o	X
7	XXX	XXX	XXX
8	o	o	X
9	o	o	X
10	X	X	X
11	o	o	o
12 control	o	o	XX

(*b*) In the next experiment serum was completely omitted and the Sachs-Georgi antigen prepared with saline diluents showing a PH reaction of 5, 6·6, 7, and 8·5.

Four tubes were arranged with contents as under:—

Tube 1 contained 0·5 c.c. antigen (diluent PH 5) and 1 c.c. saline (PH 5).

Tube 2 contained 0·5 c.c. antigen (PH 6·6) and 1 c.c. saline (PH 6·6).

Tube 3 contained 0·5 c.c. antigen (PH 7) and 1 c.c. saline (PH 7).

Tube 4 contained 0·5 c.c. antigen (PH 8·5) and 1 c.c. saline (PH 8·5).

These were placed in the water bath at 37° C., and read at the end of eighteen hours.

Tube 1 showed marked flocculation and precipitation, while tubes 2, 3 and 4 remained without change.

The PH reaction of the saline here is usually 6·6, and it is found that this reaction in no wise interferes with the performance of the test.

(3) A phenomenon which forced itself early on my notice was that whereas a well marked positive reaction might be obtained in dilutions of patient's serum 1 in 20 and 1 in 40, no reaction whatever was visible in the primary dilutions 1 in 5 and 1 in 10 when the readings were made at the end of one hour, six, eighteen, and twenty-four hours. The occurrence was relatively frequent and demanded some explanation.

Here again one was compelled to seek a parallel in the ordinary agglutinations in bacteriology. And an analogy certainly exists. It must have been the experience of every bacteriologist in the reading of results of ordinary routine agglutinations to note that when an organism is set up against increasing dilutions of patient's serum, it occasionally happens that the serum in low dilution or greater concentration fails to agglutinate the organism, while with the serum in higher dilution or less concentration marked agglutination occurs.

This phenomenon in bacteriology has been accounted for theoretically by the 'pro-agglutinoid zone,' and the terms 'zones of no reaction' and 'zones of inhibition' have been applied to those dilutions wherein agglutination fails.

Briefly the pro-agglutinoid theory consists in the belief that for various reasons (*e.g.*, length of time elapsing between the collection and examination of the blood), the agglutinins called forth by any specific agglutinogen may deteriorate or become converted into substances capable of uniting with the agglutinogens without, however, resultant agglutination.

These substances have stronger affinity for the agglutinogen than the agglutinins themselves, and are termed 'pro-agglutinoids.' If these substances, then, are present in large numbers in strongly reacting sera, they may wholly mask the reaction by preventing the actual combination of agglutinogen and agglutinin. If, on the other hand, the serum is less concentrated, then in proportion is the number of these substances so lessened that they cannot have any appreciable effect in preventing agglutinin from uniting with agglutinogen, and therefore the reaction is not obscured.

It is not, perhaps, logical to strain the similarity between the two phenomena too far when it is to be remembered that the Sachs-Georgi reaction is not even a specific antigen-antibody one, the antigen being a homogeneous suspension of lipoidal substances, and the antibody (which bears probably no relation to true antibody), a lipotrophic substance in syphilitic serum.

Similar phenomena, however, have been observed with non-specific agglutinating agents, and also in the action of coagulating agents on colloid emulsions.

Orthophosphoric acid, for example, will agglutinate a certain volume of a suspension of *B. coli* when present to the extent of between 118 cgrm. and 4 cgrm., and between 1·1 mgrm. and 0·001 mgrm., but not in intermediate amounts between 40 and 1·1 mgrm. (Hewlett).

Again, certain chemical substances have the power to agglutinate organisms, although their action is in no way specific, and the same substances will agglutinate different organisms (Beco). A mixture of equal parts of commercial formalin, alcohol, hydrogen peroxide, a 1 in 1,000 solution of chrysoidin, vesuvin, safranin, or perchloride of mercury, agglutinates the typhoid bacillus as well as other organisms. Whether, then, the phenomenon of zones of inhibition in the Sachs-Georgi reaction is determined by physical, chemical or other changes in the serum is not yet understood, but the analogy

between this and the phenomena occurring in ordinary routine agglutinations is at least very striking.

It is obvious, then, that if reliance were to be placed on the readings of the lower dilutions, or that if lower dilutions only (*e.g.*, 1 in 5 and 1 in 10) were to be put up, the results would be untrustworthy.

(*E*) CONCLUSIONS AND INFERENCES

1. The Sachs-Georgi cannot take the place of the Wassermann reaction, and should not be employed alone unless it is impossible to obtain the reagents necessary for the performance of the Wassermann.

2. The advantages claimed for the Sachs-Georgi reaction are:—

(*a*) Negligible cost of reagents and necessities.

(*b*) Simplicity of technique.

(*c*) The rapidity with which strongly reacting positive sera can be read.

3. The Sachs-Georgi, from its percentage of agreement with the Wassermann Reaction (86-89), has a quite definite value, and if strict attention be paid by laboratory workers to the following points—fallacies (which constitute the main disadvantages of the reaction) may be largely obviated, and the Sachs-Georgi may be considered at least a useful aid in the diagnosis of syphilis:—

(*a*) The patient's serum must be as fresh as possible and free from organismal contamination. If doubt exists as to sterility, cultural tests should be applied. (In this laboratory it has become routine practice to inoculate culture media tubes during the time the reactions are being performed, from all cases where the sera have been submitted from a distance and which might be likely to be contaminated. If growth occurs, the result is discarded.)

(*b*) No opinion should be given on the results of this reaction unless a detailed history of the case accompanies the serum—this with a view to exclude the co-existence of other diseases. The presence of organisms, or their

products, in the patient's blood may completely negative the value of the reaction. In cases where a patient is suffering from an acute infectious fever, the reaction should not be employed.

(*c*) The saline diluent of antigen and patient's serum must be freshly prepared, and its reaction very carefully estimated. Its reaction to PH should be 7, and a variation of not more than 6·6 to 7 allowed.

(*d*) Not less than 3 (preferably 4) dilutions of patient's serum should be put up in each series, the last tube of the series showing a dilution of not less than 1 in 40—this to obviate the fallacy dependant on the 'zones of no-reaction.'

(*e*) Final opinions should not be given until eighteen to twenty-four hours have elapsed from the time the reaction has been performed. Pseudo-flocculation, which may occur during the first few hours, tends to disappear before the end of twenty-four hours and, if still present, can be dispelled by gentle agitation of the tubes.

4. The treated cases which have been controlled throughout the full course tend to show that the Wassermann reaction remains positive longer than the Sachs-Georgi. A negative Sachs-Georgi reaction, then, in treated cases would not form a reliable index as to cure of the patient.

5. The Sachs-Georgi reaction remains negative in certain definitely established cases of syphilis, and this for no apparent reason. It is justifiable, therefore, perhaps to conclude that a negative Sachs-Georgi reaction is of little or no value.

My thanks are due to Col. G. W. Heron, D.S.O., Director of Health, for his unfailing encouragement; to my colleague, Dr. R. Briercliffe, O.B.E., for controlling those readings which may be regarded as controversial; and to Dr. K. Krikorian and Mr. K. Daghlian, of the Central Laboratory, whose assistance and lively interest in the work have rendered the production of this article possible.

NOTE ON *AËDINUS AMAZONENSIS*, LUTZ

BY
A. M. EVANS, M.Sc.

(*Received for publication* 1 *August*, 1923)

Amongst a consignment of mosquitoes collected on board s.s. 'Hildebrand' by Dr. A. Aiken Clark, during a voyage up the Amazon to Manáos, in 1922, was a male specimen of Culex with reduced palpi. It appears to be closely related to *Culex* (*Carrollia*) *paraplesia*, Dyar (1922), and comparison with the description of *Aëdinus amazonensis*, Lutz, suggests that it belongs to this little-known species.

Culex, sp. incert.

MALE. *Head.* Occiput with narrow curved creamy scales intermixed with pale golden upright forked ones above, with flat white scales at sides below and coarse golden setae projecting forward round eye margins. Palpi slender, about one-seventh the length of the proboscis, vestiture of pale brown scales. Proboscis bent at outer two-thirds, expanding distally (these two conditions probably due to accident), scales blackish brown, labellae yellowish. Antennae densely plumose, hairs of whorls blackish brown.

Thorax. Prothoracic lobes pale ochraceous, with a few flat whitish scales above and a row of blackish setae. *Mesonotum* with integument bright ochraceous, irridescent; in certain lights a narrow median dark stripe visible; vestiture of hair-like, bronze-coloured scales, with paler reflections, the scales in front of ante-scutellar space paler. *Scutellum* pale brown, with pale scales.

Abdomen. Dorsally blackish-brown scaled, with minute creamy triangular lateral spots on the last two segments. Venter largely denuded.

Legs with vestiture of blackish-brown scales.

Wings with first fork cell long and narrow, about four times as long as its petiole; second fork cell about twice the length of its

petiole. Scales on distal half of wing mostly of the type illustrated in the accompanying figure (fig. 1 A) widest beyond the middle, and with rounded apex. Scales on proximal half short, broad and truncated.

Hypopygium (fig. 2). Side-pieces (fig. 2 A) closely resembling those of *C.* (*Carrollia*) *paraplesia*, Dyar, but with a greater number of stout spines between the lobe (*l.*) and the clasper; apical part of lobe missing. *Mesosome* (fig. 2 B, C and D): lower bridge with small, highly chitinised, finely setose area (fig. 2 D, *b.*). Halves of mesosome consisting of thin plates of the form shown in the figures (fig. 2 B and C). In dorsal aspect the proximal portion (*p.*) is seen to give rise to a short inner portion (*i.*) and a much longer outer portion (*o.*), which constitutes the main part of the mesosome plate as seen in lateral view. *Ninth tergites* (fig. 2 E) about twice as high as broad; *tenth sternites* comb-shaped, with eight teeth.

It has recently been suggested by Dyar (1923) that the specimens described as *Culex originator*, Gordon and Evans (1922), from the Amazon Region, represent *Aëdinus amazonensis*, Lutz. I have, therefore, compared the types of *C. originator* with the description of Lutz's species and find that they differ in the colour of the thoracic integument, which is dark grey as described, and not ochraceous as in *A. amazonensis*; and in the character of the scales of the mesonotum and wings. The thoracic scales are narrow, but not hair-like as they are said to be in *A. amazonensis*, and there are no scales on the wings which could be described as 'Taeniorhynchus-like.' The main types of scales found on the apical part of the wing are illustrated in fig. 1; the scales on the proximal half of the wing are short and truncated. The specimen described above, however, agrees with *A. amazonensis* in these three particulars, the thoracic integument being of a conspicuously ochraceous colour. The only noteworthy difference appears to be the absence of a well defined median and fainter lateral dark mesonotal stripes in my specimen, which only shows a faint median stripe in certain lights. If, however, this character be subject to variation, it would appear highly probable that this Culex is *A. amazonensis*.

The structure of the side-pieces would seem to indicate a very close relationship with *Culex* (*Carrollia*) *paraplesia*, Dyar, but the latter differs in other hypopygial characters, the tenth sternites

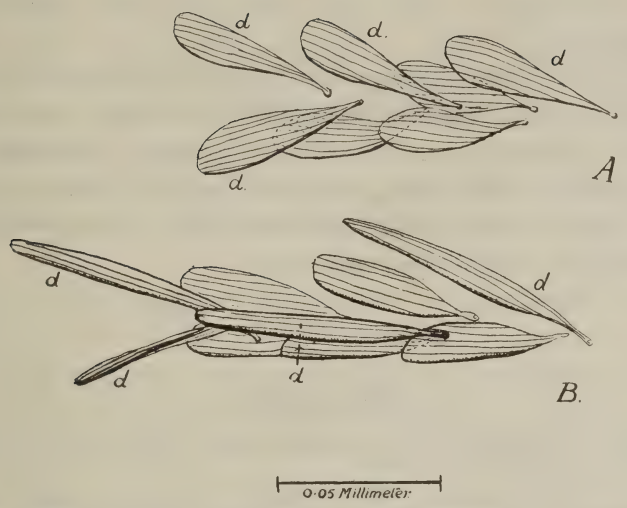

Fig. 1. Scales from upper branch of vein II. A.—*Culex* sp. incert; B.—*Culex originator*, Gordon and Evans. *d.*—scales of upper surface of wing.

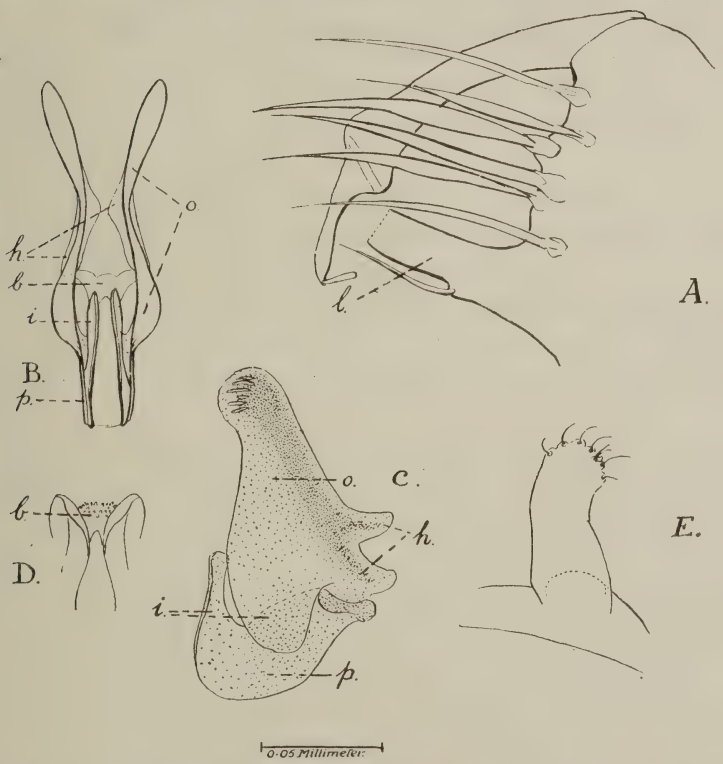

Fig. 2. *Culex* sp. incert. Hypopygium. *A.*—Side piece, in part with clasper. *l.*—lobe, broken distally. *B.*—Mesosome from above. *b.*—horns; *i.*—inner, *o.*—outer, *b.*—lower bridge.

having only three or four teeth, and the ninth tergites being undeveloped.

It is thus seen that at least two species of Culex with reduced male palpi occur in the Amazon Region; possibly others may be discovered. Should one be found agreeing externally with *A. amazonensis*, and having marked thoracic stripes as in that species, I would suggest that the name *Culex hildebrandi* be used to designate the species described above.

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THE TREATMENT OF AMOEBIC DYSENTERY

BY
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The majority of the cases recorded in the following paper were treated as in-patients at the Tropical Ward of the Royal Infirmary, Liverpool, their subsequent history after discharge being followed at the Tropical Clinic in the same city. At first it was hoped that much information might be gained by consulting the hospital and other records of the Ministry of Pensions, and through the courtesy of Dr. Finlay and others some four hundred case sheets of amoebic dysentery patients were examined. The results obtained were disappointingly meagre ; a few of the cases are included in the tables that follow, but in the majority of instances the observation periods after treatment were too short to test the value of the drug given.

The following definitions were adhered to throughout :—

(1) The first diagnosis of amoebic dysentery was made by the finding of motile amoebae containing red cells in the faeces.

(2) Such a patient was considered to have relapsed after the completion of treatment when diarrhoea again occurred and active amoebae were observed in the stool, blood and mucus being usually, though not always, present.

(3) Cases which after treatment passed *E. histolytica* cysts unaccompanied by motile amoebae were not considered to have relapsed, but all such instances are recorded in the tables under the heading " Remarks."

(4) Once a patient relapsed he was considered as a " fresh case " and any other course of treatment was placed under a separate entry.

While undergoing treatment and for the first fortnight after treatment the stools were usually examined twice weekly (sometimes, as in the case of the emetine periodide series, much oftener) during the remainder of the observation period the examinations averaged about one a fortnight. In every instance, tests for the presence or absence of amoebae in the

stools were made by some member of the Liverpool School of Tropical Medicine.

No attempt has been made to compare the value of any two forms of treatment, as the observation period was not constant. Thus the relapses in treatments I and II were respectively 84 and 75 per cent., but if we fix an arbitrary limit of one month's observation and disregard all relapses occurring at a later date, then the relapses become respectively 53 and 17 per cent.

Note on cases treated with emetine periodide. Willmore (1923) records ninety-one cases of amoebic dysentery treated with emetine periodide of whom forty-eight (52 per cent.) relapsed. His observation period is similar to that used in the present paper, but his definition of a relapse includes persons passing *E. histolytica* cysts after treatment. Applying this definition to the sixteen cases recorded in Table I, treatment No. IX shows that ten of the sixteen cases (62 per cent) relapsed. Various vehicles for administering the drug were tried; formalised gelatin capsules were given in two cases of the acute type and all the motions passed in the subsequent twenty-four hours saved, by this method it was found that in both instances the capsules were passing through intact; even the plain gelatin capsules administered to patients with diarrhoea frequently passed through the gut without dissolving. Rice paper cachets were excellent, but owing to their brittle character sometimes allowed part of their contents to escape. At present we give the drug mixed with a little milk; taken this way it sometimes causes slight nausea but never vomiting. As Willmore's cases were all of the type that had ' proved refractory to all the known standard methods of anti-amoebic treatment ' it appears of interest to record two cases of acute dysentery, one which (H.J.C.) had never received previous emetine treatment. Amoebae disappeared from this man's stool within forty-eight hours of the start of treatment and did not reappear during the six weeks he was kept under observation. The other case (C.M.P.) had received only one previous course of emetine (twelve grains emetine hydrochloride given fifteen months previously). During the first six days of treatment this patient continued to pass blood, mucus and amoebae, and at the end of this time his condition was so bad that it was thought advisable to supplement the periodide with four hypodermic injections of emetine hydrochloride. Under this combined treatment the amoebae vanished from the stools within twenty-

TABLE I.

Showing the effect of various forms of treatment on one hundred and thirty-eight cases of amoebic dysentery.

Nature of treatment		Observation period in months after completion of treatment							Total	Remarks
		1	2	3	4	5	6	more than 6		
ATMENT No. I. Showing the effect of tment with emetine rochloride gr. 1 given sub-neously or intramuscularly wo to six consecutive days.	Relapsing	7	4	...	...	...	...	...	11	One non-relapsing case was passing *E. histolytica* cysts four months after completion of treatment. Most of the cases in this series were of the acute type, i.e. passing a large number of blood and mucus motions in the 24 hours.
	Not relapsing	1	...	...	1	...	...	...	2	
ATMENT No. II. Showing the effect of tment with emetine rochloride gr. 1 given sub-neously or intramuscularly en to fourteen consecutive .	Relapsing	5	4	1	3	...	2	6	21	One non-relapsing case was passing *E. histolytica* cysts 12 months after completion of treatment. The majority of the cases were of the acute type. Two non-relapsing cases were observed for more than a year.
	Not relapsing	1	2	...	2	...	...	2	7	
ATMENT No. III. Showing the effect of tment with emetine uth iodide grs. 3 given by th on twelve or thirteen ecutive days.	Relapsing	10	4	3	...	2	...	6	25	Most of these cases were of the chronic type, i.e. passing a daily average of four to six loose stools containing active amoebae but little or no blood or mucus.
	Not relapsing	3	2	1	...	...	1	...	7	
ATMENT No. IV. Showing the effect of ment with emetine ochloride gr. 1 given sub-neously together with ine bismuth iodide gr. 1 1 by the mouth on twelve ecutive days. (Three cases ten days).	Relapsing	11	...	2	1	...	1	1	16	Two non-relapsing cases were passing *E. histolytica* cysts, respectively, 12 and 20 months after completion of treatment. The cases include about an equal proportion of acute and chronic types. Three non-relapsing cases were observed for 18 months.
	Not Relapsing	1	1	...	...	...	2	4	8	

TABLE I.—*continued.*

Showing the effect of various forms of treatment on one hundred and thirty-eight cases of amoebic dysentery.

Nature of Treatment		Observation period in months after completion of treatment.							Total	Remarks
		1	2	3	4	5	6	more than 6		
TREATMENT No. V. Showing the effect of treatment with pulv. ipecac. grs. 5, together with pulv. ipecac. Co. grs. 5 given by mouth on thirty consecutive days.	Relapsing	2	...	...	...	...	1	2	5	The majority of these c were of the chronic type.
	Not relapsing	...	1	1	...	...	...	1	3	
TREATMENT No. VI. Showing the effect of treatment with pulv. ipecac. grs. 5, together with pulv. ipecac. Co. grs. 5 given by mouth on sixty consecutive days.	Relapsing	2	...	...	...	...	...	...	2	Most of these patients w of the chronic type and l repeatedly relapsed un various other forms of tre ment.
	Not relapsing	1	...	...	1	...	2	3	7	
TREATMENT No. VII. Showing the effect of treatment with Ravauts paste, three drachms given by mouth on thirty consecutive days.	Relapsing	...	...	...	2	...	...	...	2	These were mild cases a chronic type.
	Not relapsing	1	...	...	...	...	...	...	1	
TREATMENT No. VIII. Showing the effect of treatment with Ravauts paste, three drachms given by mouth on sixty consecutive days.	Relapsing	3	...	...	...	...	...	1	4	These were mild cases a chronic type.
	Not relapsing	...	...	...	...	...	1	...	1	
TREATMENT No. IX. Showing the effect of treatment with emetine periodide grs. 6 given by mouth on thirteen to fifteen consecutive days. In one case, which is included amongst the non-relapsing, the emetine periodide was supplemented with four injections of emetine hydrochloride gr. 1.	Relapsing	8	...	...	...	...	...	...	8	Two non-relapsing cases w passing *E. histolytica* cy respectively, two and six we after the completion of tre ment. The cases include ab an equal proportion of ac and chronic types.
	Not relapsing	1	2	2	2	1	...	...	8	

four hours and no relapse occurred during the five months the patient was kept under observation. Two other cases who had received numerous previous courses of emetine continued to pass motile amoebae throughout the time of treatment. Daily examinations of the remaining twelve cases showed that in two of them motile amoebae persisted for five days of treatment and in the other ten cases vanished after one to three days.

TABLE II.

Showing effects of various treatments not recorded in Table I.

Treatment.	Number of cases treated.	Result of treatment.
Emetine hydrochloride ½ gr. given subcutaneously on two consecutive days.	1	Relapsed after six days.
Emetine hydrochloride ⅓ gr. given subcutaneously on twenty-two consecutive days.	1	Relapsed seven months later.
Emetine hydrochloride ⅓ gr. given subcutaneously on thirty consecutive days.	1	Relapsed within a month.
Emetine hydrochloride gr. 1 given subcutaneously together with emetine hydrochloride ½ gr. given by mouth on ten consecutive days.	2	(1) No relapse after three months observation. (2) No relapse after three months observation.
Emetine hydrochloride gr. 1 given subcutaneously on six consecutive days followed by emetine bismuth iodide grs. 3 on twelve consecutive days.	2	(1) No relapse after twelve months observation. (2) Relapsed six months later.
Emetine hydrochloride gr. 1 given subcutaneously on twelve consecutive days followed by emetine bismuth iodode grs. 3 given by mouth on six consecutive days.	1	No relapse after twelve months observation.
Emetine hydrochloride gr. 1 given subcutaneously together with emetine bismuth iodide grs. 3 given by mouth on six consecutive days.	2	(1) No relapse after six weeks observation. (2) Relapsed a week later.
Emetine bismuth iodide gr. 1 given by mouth on twenty-four consecutive days.	1	Relapsed within a month.
"Yatren" 200 ccs. of a 5 per cent. solution given per rectum on ten consecutive days, then six days rest followed by a like dose for one day only.	1	Relapsed within a fortnight.

Note on case treated with Yatren. Mühlens and Menk (1921) recommend ten grammes of Yatren given by the rectum for eight to fourteen days, then no treatment for seven days; repeat the Yatren for three to seven days, allow another resting period of seven days and repeat treatment for three to five days. Owing to the fact that only a limited quantity of the drug was available, the course was shortened to that shown in Table II.

TABLE III.

Showing the distribution in various months of the numbers and percentages of one hundred and one relapses after treatment.

Treatment.	Relapses	Month in which relapse occurred after completion of treatment.						
		1	2	3	4	5	6	More than 6
Emetine hydrochloride gr. 1 given subcutaneously or intramuscularly on two to six consecutive days.	Number	7	4	...	...	...	...	...
	Percentage	63	36	...	...	...	...	...
Emetine hydrochloride gr. 1 given subcutaneously or intramuscularly on ten to fourteen consecutive days.	Number	5	4	1	3	...	2	6
	Percentage	23	19	4	14	...	9	28
Emetine bismuth iodide grs. 3 given by mouth on twelve to thirteen consecutive days.	Number	10	4	3	...	2	...	6
	Percentage	40	16	12	...	8	...	24
Emetine hydrochloride gr. 1 given subcutaneously together with emetine bismuth iodide gr. 1 given by mouth on twelve consecutive days (three cases only ten days).	Number	11	...	2	1	...	1	1
	Percentage	68	...	12	6	...	6	6
Pulv. ipecac. grs. 5 together with pulv. ipecac. Co. grs. 5 given by mouth on thirty consecutive days.	Number	2	...	...	...	...	1	2
	Percentage	40	...	...	...	...	20	40
Pulv. ipecac. grs. 5 together with pulv. ipecac. Co. grs. 5 given by mouth on sixty consecutive days.	Number	2	...	...	...	...	...	...
	Percentage	100	...	...	...	...	...	...
Ravauts paste three drachms given by mouth on thirty consecutive days.	Number	...	...	...	2	...	...	...
	Percentage	...	...	...	100	...	...	...
Ravauts paste three drachms given by mouth on sixty consecutive days.	Number	3	...	...	...	...	...	1
	Percentage	75	...	...	...	...	...	25
Emetine periodide grs. 6 given by mouth on thirteen to fifteen consecutive days (One case supplemented with four injections of emetine hydrochloride gr. 1).	Number	8	...	...	...	...	...	...
	Percentage	100	...	...	...	...	...	...
Various treatments recorded in Table II.	Number	5	...	...	...	...	1	1
	Percentage	71	...	...	...	...	14	14

The patient (A.G.) was a chronic case of about five years' duration who had completely resisted, or else relapsed shortly after, numerous forms of treatment. Before treatment commenced he was passing eight to ten motions a day containing blood, mucus and active amoebae. Twenty-four hours after the first rectal injection the amoebae disappeared and the number of stools were reduced to one or two a day. This condition lasted for twenty-eight days when blood, mucus and amoebae again appeared in the faeces. A further supply of the drug has been obtained and other patients are now under treatment.*

SUMMARY

One hundred and fifty cases of amoebic dysentery were given various forms of treatment and subsequently kept under observation for one to six months or longer; of these one hundred and fifty cases, one hundred and one (66 per cent.) relapsed, the numbers and percentages of the relapses occurring in various months being recorded in the tables. Amongst all the cases treated only six (4 per cent.) were observed to be passing *E. histolytica* cysts after treatment. Sixteen cases were given emetine periodide grs. 6 daily, eight of these (50 per cent.) relapsed within one month; the giving of this drug in gelatin capsules was found to be unsatisfactory as they frequently passed through the gut without dissolving; the periodide when mixed with a little milk and given by the mouth did not produce vomiting. Owing to the inequality of the observation periods no attempt was made to compare the value of any two forms of treatment.

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* Since the publication of this note another case has relapsed, fourteen days after the completion of treatment. The patient in this instance had received the full course of treatment recommended by Mühlens and Menk.

RELAPSING FEVER IN THE GOLD COAST

BY

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WEST AFRICAN MEDICAL STAFF

(*Received for publication* 20 *August*, 1923)

The following account of an outbreak of relapsing fever in Accra, Gold Coast Colony, has been considered worthy of record since the disease has never before been recognised in the Colony.

Doubts have been expressed as to the possibility of the disease having been prevalent in the Colony in former years. For it is well known that circumstances tend to render the recognition of the disease unusually difficult since attacks may be so mild in character as to resemble slight attacks of malaria. Examples of this nature were met with during the outbreak to be described, and the danger of spread resulting from the movements of persons suffering from the ambulatory type of spirillar fever was readily appreciated. It is difficult, however, to understand how the disease could have existed undetected for, as can easily be understood, routine blood examinations in all cases of fever can be numbered in their hundreds every year, and had a proportion of these unclassified fever patients been suffering from relapsing fever, the spironema could scarcely have escaped detection.

In connection with this it should be stated that Simpson discovered spironemata in a single blood smear out of a large number of blood smears examined in 1908, the year of an outbreak of plague in the Gold Coast Colony.

EPIDEMIOLOGY

Two possible sources of infection present themselves in connection with the epidemic to be described hereafter. One of these appears to have been the soldiers who returned to the colony after hostilities had come to an end in what is now called Kenya Colony. It is recorded that the West African forces, especially those in the

Dar-es-Salaam area, suffered severely from relapsing fever. It is possible that a certain number remained infective subsequent to their return to the colony and that infection spread to their families and to neighbouring tribes.

This theory is supported to some extent by the fact that the majority of the returned troops were members of the Northern Territory tribes and that many of these took their discharge from the Headquarters of the West African Frontier Force at Coomassie. The theory gains further support from the large preponderance of Northern Territory tribesmen, some of whom had seen war service, amongst the patients encountered during the outbreak at Accra. Moreover, a report has recently come to hand from the Medical Authorities in the Northern Territories of the Colony, to the effect that six cases of relapsing fever (confirmed by blood examination) have been isolated from recruits to the West African Frontier Force.

There are, however, several excellent objections to the acceptance of this theory, not the least of which is the fact that the West African troops while in East Africa suffered from infection by *Sp. duttoni* carried by the *Ornithodorus moubata*, whereas the strain met with in the Accra epidemic resembled *Sp. obermeieri*, in this instance the vector being the body louse.

The other possible source of infection may have been the neighbouring French territory, since a very extensive epidemic of relapsing fever was reported from Senegal and French Niger Territory in 1921.

Epidemics of relapsing fever are said to be slow in onset and to show a gradually increasing mortality. In the outbreak under review the onset was certainly rapid though its rapidity is slightly obscured in the chart (No. 1) owing to the artificial conditions resulting from emergency legislation. The mortality, moreover, fortunately showed no gradual increase in severity, as seen by the fact that the case mortality rate for the first fifty cases was 6 per cent., while that for the remainder was less than 1 per cent.

INCIDENCE

(*a*) *Sex*

As in India and other countries where the disease is endemic the large majority of cases occurred in adult males, the actual figures being one hundred and fifty-six male cases (including two European cases) and only two female cases.

This very large preponderance of male cases is not dependent upon the degree of lousiness of the two sexes, for observations showed that infestation was shared in equal measure by both men and women. The explanation, rather, lies in the fact that the infected males belonged almost exclusively to an immigrant tribe, who had come into the Accra district from their Northern Territory villages in search of work and money to purchase European goods. It is contrary to the customs and habits of these tribesmen to bring their women and children with them, and as they return to their native villages as soon as they have been able to collect together a small quantity of trade goods, they do not possess property and housing accommodation in Accra. It is not difficult to understand the conditions under which these people live in Accra, crowded together in insanitary hovels lacking in light and air.

(*b*) *Age*

Since the majority of cases of relapsing fever occurred amongst males of Northern Territory tribes, it follows that most of the cases would occur in adults, since the journey to Accra from the Northern Territories is not a thing to be lightly undertaken by persons other than healthy adults. The age of the patients varied from 10 years in a Hausa boy to 55 in a Zabramah man. The patients were almost all illiterate and consequently their ages could only be estimated approximately—the average being between 25-30.

(*c*) *Race*

The following table gives the racial incidence.

Table I.

Race or Tribe	Number of Cases	Percentage
European	2*	1·2
Kroo	2†	1·2
Kotokoli	3	1·8
Hausa	3	1·8
Other Tribes	9‡	5·6
Zabramah (Zaberrima)	139	87·9
Total	158	99·5

* A European who became infected in the course of experiments, and who became infected a second time at a later date, is included in this figure.

† Two volunteers who were infected in the course of experiments.

‡ Includes a volunteer who was infected in the course of experiments as in the other three cases.

The reasons for the preponderance of Zabramah and other Northern Territory tribesmen amongst the cases are not hard to seek. Reports of extensive epidemics of doubtful character are not infrequently received from the Northern Territories and one such, accompanied by heavy mortality and attributed to cerebro-spinal meningitis took place in 1920. It is possible that relapsing fever may have been the cause of certain of these outbreaks and that shortage of medical staff allowed it to remain unrecognised. In any case an undoubted epidemic of relapsing fever occurred amongst natives in French Territory bordering upon the Northern Territories in 1921, and intercommunication across the frontier would account for infection.

Once infected, the habits of these tribes would ensure a rapid spread and perpetuation of the infection. Owing to a great scarcity of water in most parts of the Northern Territories, except in the wet season, the average tribesman from this area is brought up from birth in the belief that water is intended for drinking and cooking purposes only. In consequence his body, clothes, bedding and living quarters are quite innocent of soap and water.

This condition of affairs contributes to a state of lousiness, and infestation with lice is so general (100 per cent. of the Northern Territory patients admitted to the Contagious Diseases Hospital, Accra, were found to be lousy on admission) that little, if any, attempt at disinfestation is made on the part of sufferers from lice. Coastal tribes in the Contagious Diseases Hospital were amazed at the Health Authorities interfering with what was considered a 'custom of the country' when disinfestation of admissions was carried out. The fact that Northern Territory tribesmen and Hausas sleep in their work-a-day garments tends to add to the degree of natural lousiness.

In this connection it is a remarkable fact that coastal tribes, not excepting the Kroos, are singularly free from lice, due, no doubt, to the fact that they do not share the aversion from washing their bodies and clothes exhibited by Northern Territory tribesmen. In order to obviate the possibility of the importation of plague, smallpox and other infectious and contagious diseases into the Colony, all Kroo immigrants from the Kroo coast are medically examined on arrival at this port. Nine hundred and thirty-one were

so examined in 1922, and although they had been in most cases crowded together on board ship and had not had facilities for washing their bodies and clothes for as long as three weeks in some cases, not a single one was found to be lousy.

A further reason for the large proportion of Northern Territory tribesmen among the cases of spirillar fever lies in the insanitary conditions in which they lived in Accra. Not possessing any house property or relatives with satisfactory living accommodation, they crowd together in insanitary corrugated-iron structures lacking light and ventilation, intended by their unscrupulous owners not as living quarters but as stores for building materials and the like.

Lastly, owing to a temporary trade depression many of the Zabramahs were unemployed for some time prior to the commencement of the outbreak and, having neither friends nor relations in the district, they became half starved. The synonym 'famine fever' indicates the traditional association of semi-starvation and relapsing fever. With their powers of resistance so diminished they afforded fertile soil for the germ of any infectious disease.

The number of cases occurring amongst other tribes was too small to warrant conclusions being drawn as to the comparative severity of the disease, but it can be stated with fairness that the more severe type of case—and in fact all the fatal cases—occurred amongst Zabramahs.

(*d*) *Case Distribution*

A map is appended showing the districts in which patients appeared to have been infected.

Neglecting the first case which occurred in a European who had passed through a considerable number of bush villages in the Accra District during the course of his work, a number of the earlier patients appeared from information obtained from them to have been infected either in their native villages in the Northern Territories, or in one of the towns or villages at which they rested at night time, during their journey to Accra. This bears out the theory that the epidemic in Accra and District originated directly in Northern Territory tribes.

It will be seen from the map that the vast majority of cases occurred in the Tudu and Zongo Rd. areas of Accra, in both of which the inhabitants are almost entirely members of Northern

TABLE II.

Table to show location of places where patients are presumed to have been infected.

Place	Number of Patients infected
Accra	130*
Some village in Accra bush or on the road from the Northern Territories to Accra	19†
Nsawam	6
Mangoase	3
	158

* Includes three African patients accidentally infected in the Colonial Hospital, and three Africans and one European infected experimentally at the Medical Research Institute (the European suffered from two attacks).

† Includes one European case infected in some unknown village in the Accra bush.

TABLE III.

Table to show areas in which cases of relapsing fever occurred, but not necessarily where infection took place.

	Number of Cases
Accra, Block No. 11	1
Accra, Block No. 12	19
Accra, Block No. 13a	43
Accra, Block No. 15a	8
Accra, Block No. 15b	50
Accra, Block No. 16	13
Accra, Block No. 17	3
Accra, Medical Research Institute	4*
Accra, Native Hospital	3
Accra Bush	6†
Residence unknown	8
Total	158

* These cases were infected experimentally.

† This number includes a European, the first case to be discovered at the commencement of the epidemic.

— ACCRA —

— TO SHOW WHERE CASES OF RELAPSING FEVER —

— OCCURRED DURING THE EPIDEMIC —

— MARCH TO JULY 1923 —

Territory tribes or Hausas—both of whom live under unhealthy conditions.

Four cases are shown to have been infected during the course of experiments aiming at the discovery of the means of transmission of the spironema, while three cases are shown as having been infected in the general wards of the Native Hospital, in which a number of relapsing fever patients were treated, prior to the declaration of the disease as an infectious disease necessitating removal to the Contagious Diseases Hospital.

It is noteworthy in connection with the distribution of cases that the areas from which they came were of a very low standard as regards housing accommodation, overcrowding in insanitary, ill-lighted and ill-ventilated hovels.

(*e*) *Mode of Transmission of the Disease*

During the initial stages of the Accra outbreak of spirillar fever preventive measures against the spread of the disease were greatly hampered by the lack of knowledge of the exact means of transmission of the organism.

Ornithodorus moubata Murray, the carrier of tick fever in the Congo, and *Ornithodorus savignyi* Aud, the possible carrier of relapsing fever in Somaliland, have not yet been recorded as occurring in the Gold Coast. *Ornithodorus talaje* Guérin-Méneville, the carrier of relapsing fever in Panama, was taken by Graham on *Cricetomys gambianus* (Report on Plague in the Gold Coast in 1908: W. J. Simpson, p. 22), it was also taken on *M. decumanus* during the small epidemic of plague in Accra in 1917, but its occurrence is so rare as to preclude its being the prevalent carrier of the present outbreak of relapsing fever. *Argas persicus* Oken is supposed by some inhabitants of the Gold Coast to be a common parasite of fowls in the Colony, but there is no authentic record of its occurrence; moreover, it has been shown by Edm. Sergent and H. Foley (1922) that this tick is not a carrier of North African relapsing fever, whereas *Pediculus humanus* is an efficient one.

Feeding experiments were carried out with bed bugs, mosquitoes, and lice.

Bed bugs

On the 14th April seven bed bugs collected from the clothes and

bedding belonging to cases of relapsing fever which had been interned in the Contagious Diseases Hospital, were placed on the shaven back of a small monkey. At least two of these bugs fed on the monkey, but it was difficult to keep them in position so that the experiment was abandoned within four hours of its commencement. The monkey during the following fortnight showed no symptoms of illness and spironemata were never found in its blood.

A second consignment of bugs from the same source numbering a dozen was placed on the arm of a native volunteer on the 15th April, a modification of Nuttall's pill-box method of feeding lice being adopted. The bugs were retained on the arm for three days and were then removed and dissected, no spironemata were found in them and the volunteer never showed any symptoms of relapsing fever.

On the 30th April twenty bugs which had been collected from the bedding of relapsing fever cases were kept alive in the incubator for four days and were then ground up in saline. The coarser particles having been removed, the emulsion, which contained no spironemata, was rubbed into the scarified skin of a volunteer; the volunteer never showed signs of relapsing fever and spironemata were not detected in his blood at any time during the two weeks following the inoculation.

Mosquitoes.

Aedes argenteus Poiret (*S. fasciata*) being probably the most universally distributed mosquito in West Africa, and being the recognised carrier of *Leptospira icteroides*, was selected for experiment. Eleven female *Aedes argenteus*, reared from larvae, were placed in a gauze-covered jar and fed upon the arm of a patient suffering from relapsing fever in the Contagious Diseases Hospital before the patient had received any treatment. Five of the mosquitoes were seen to be engorged with blood when they were brought back to the laboratory on the 13th April. The mosquitoes were separated into two lots; six mosquitoes, of which three had certainly sucked blood, were placed in one jar and five, of which two were engorged with blood, were placed in another jar, honey and water were placed in these jars and the mosquitoes were kept alive with occasional renewal of the honey until the 24th April. Two volunteers began to feed these mosquitoes on their arms on that date

—(one jar being assigned to each volunteer)—and continued to feed them daily till the 2nd May when the mosquitoes began to die. No spironema was found in any of the surviving mosquitoes on dissection and neither of the volunteers showed symptoms of relapsing fever during the fortnight following the abandonment of the experiment, and at no time were spironemata found in the blood of either of them.

Lice. (*Pediculus humanus*).

On the 14th April four lice (*Pediculus humanus*), collected from the clothing of a case of relapsing fever, were placed by the aid of the pill-box method on the back of a monkey, but they refused to suck blood and were therefore transferred to the arm of a native volunteer: on the 17th April these lice were all found to have died. They were at once ground up in saline and the emulsion was inoculated into a black rat. Neither the volunteer nor the black rat showed any signs of illness and no spironemata were at any time found in the blood of either.

On the 30th April twelve lice were placed on the arm of a native volunteer; these lice had been found in the clothing of contacts with relapsing fever cases, nine of the lice were found to be dead on the following morning. The three surviving were then placed on a case of relapsing fever in the Contagious Diseases Hospital; after sucking blood from this case they were brought back to the laboratory, kept in the incubator for forty-eight hours, and were then placed on the arm of a second volunteer who managed to keep them alive for three days. Neither of the volunteers suffered in the least degree during the two weeks succeeding the feeding of the lice and neither showed spironemata at any time in his blood. The nine lice from this batch which were found dead on the arm of the first volunteer on the 1st May were ground up in saline and the resulting emulsion injected subcutaneously into a monkey without any effect following, the monkey remained well and spironemata were never seen in its blood.

On the 14th May a dozen lice obtained from the clothing of a case of relapsing fever in the Contagious Diseases Hospital were placed on the arm of a native volunteer and left *in situ* for seventy-two hours. At the end of that time ten were found dead, the two survivors together with the ten dead were ground up in saline and

the emulsion inoculated subcutaneously into a monkey. Neither the volunteer nor the monkey developed symptoms and spironemata were not found in the blood of either during two weeks following the experiment.

On the 16th May twenty-five lice were received from the Medical Officer of Health who had collected them from the clothing of cases of relapsing fever. These lice were divided into two lots and fed on the arms of two volunteers for three days with the same result as in previous experiments—neither volunteer developing symptoms of relapsing fever or at any time showing spironemata in his blood.

The droppings from these lice adherent to the sides and bottom of the test tube in which they were received were gently washed out with saline and the mixture rubbed into the scarified arm of a volunteer, but he developed no symptoms and his blood remained free from spironemata for two weeks following the inoculation.

On the 26th May two dozen lice collected from the clothing of several cases of relapsing fever in the Contagious Diseases Hospital were received and were placed on the arm of one of us (A.I.) by the aid of the pill-box method; these lice were fed at intervals during the day and night till the 4th June when, there being only six survivors, they were ground up in saline and the emulsion which contained spironemata was inoculated into the scarified arms of two native volunteers. On the 12th June one of the volunteers complained of headache and lumbar pain, his temperature was found to be 100·2°F. and spironemata were found in thin and thick films of his blood. On the 13th June the other volunteer reported sick, his temperature was 100·6°F. and his blood showed spironemata. No spironemata were present in the blood of the individual upon whose arm these lice were placed at any time; it was examined from the 26th May to the 4th June whilst the lice were being fed.

On the 4th June four lice were supplied by the Medical Officer of Health (P.S.S-C.). These lice had been allowed to remain on a patient suffering from relapsing fever for five days after he had been treated with Novarsenobillon. When received at the laboratory the lice were placed on the arm of a native volunteer using the modified pill-box method; they were left on this individual's arm for twenty-four hours only and one of them had disappeared when the pill-box was removed. The three survivors were ground up in

saline and a portion of the emulsion was rubbed into the scarified arm of another volunteer, a second portion was dropped into the conjunctival sac of a monkey, while the residue was inoculated subcutaneously into a second monkey. This emulsion contained spironemata. Neither of the monkeys became ill and during the two weeks following the experiment no spironema was found in the blood of either. The second volunteer into whose arm the emulsion had been rubbed suffered from headache, and had a temperature of 99·2°F. on the 14th June, but no spironemata were found in thick films of his blood examined daily for a week after the slight rise of temperature. The first volunteer upon whose arm the four lice were placed began to be ill on the 15th June, but did not report himself as sick until the 18th June, when his temperature was found to be 102°F. and spironemata were numerous in thick films of his blood.

On the 11th June twenty lice from the clothing of cases of relapsing fever in the Contagious Diseases Hospital were supplied by the Medical Officer of Health (P.S.S-C.); these were at once placed on the arm of a native volunteer who fed them for three days. On the 14th June, however, as he seemed reluctant to continue to feed the lice, the survivors, five in number, were transferred to the arm of one of us (A.I.) where they were fed for another four days. During the last period of feeding two of the lice escaped one night from under the bandage retaining the pill-box in position and wandered freely over the body—judging from the number and position of the bites discovered the following morning. These lice were not recovered and may have been crushed in an attempt to allay the irritation of their bites. On the 18th June the feeding of the three ultimate survivors was discontinued and they were ground up in saline and the resulting emulsion, which contained spironemata, was inoculated subcutaneously into a monkey. This monkey showed no symptoms of illness and spironemata were not at any time found in its blood, which was examined daily till the 2nd July. The native volunteer who fed this batch of lice for three days immediately following their last meal of infective blood remained free from sickness, and no spironemata were found in his blood which was examined daily for twelve days after he had discontinued feeding the lice. The second individual who continued the feeding of the

lice became ill on the 25th June, his symptoms were headache and pains in the limbs and his temperature was 101·6°F., but no spironemata were found in a thick film of blood; on the morning of the 26th June when the temperature was 102.4°F. a few spironemata were seen in a thick film of blood, they became more numerous at a later stage of the attack.

It may be of interest to mention that the individual who became sick as the result of this last feeding experiment was the same who developed relapsing fever as a result of accidental inoculation on the 28th March. It is possible that this second attack may have been a relapse of the former, but it appears to us more probably a reinfection, the interval between the recovery from the first attack and the onset of the second, a period of eleven weeks, being too great; besides at no time during the interval were any spironemata found in the blood which was repeatedly examined, and no symptoms of illness were experienced.

These feeding experiments appear to agree with the conclusions of the French observers in Tunis and Algeria, Ch. Nicolle, L. Blaziot et E. Conseil (1912), and Edm. Sergent et H. Foley (1922), namely, that the disease is not conveyed by the bites of lice or by their droppings being rubbed into excoriations of the skin, but that it is conveyed by the inoculation of crushed lice into wounds of the skin; further, that lice must be kept alive for about one week after feeding on a case of relapsing fever before they are capable of conveying the infection.

The two last experiments may seem rather equivocal, suggesting that the infection is conveyed by the bites of lice alone. It is to be noted, however, that in both experiments one or two lice escaped from the pill-box in which they were enclosed and wandered over the body generally; it is therefore quite possible that these stray lice were unconsciously crushed and rubbed into abrasions of the skin made in the process of scratching.

The pill-box method of feeding may not be an ideal method for use in the tropics, as pointed out by Cragg (1922), but when properly applied it certainly prevents crushing of its contents on the skin by any attempts at scratching.

Up to the time of writing, experiments have not been carried out to show whether infection is transmitted by infected lice to their

eggs, though this has been shown to be the case in other parts of the world.

It is noteworthy that lice were found on a very large proportion of all the patients treated at the Contagious Diseases Hospital, more especially among Zabramahs and other Northern Territory tribes and Hausas, in whom lice were found on the hair of the head, beard, axillae, pubic region and on wearing apparel.

The louse appeared to resemble the head and body louse found in Europe—*Pediculus humanus L.* It was remarked that lice tended to migrate from an individual having a high temperature, suggesting that the optimum skin temperature was probably in the neighbourhood of 98·4°F. or less. The temperature of the air and the relative humidity did not appear to influence the numbers or habits of the louse.

A graph and table given in the appendix illustrates the lack of influence exercised by temperature and humidity on the numbers of cases discovered in Accra from week to week.

(f) Time of Occurrence of Cases

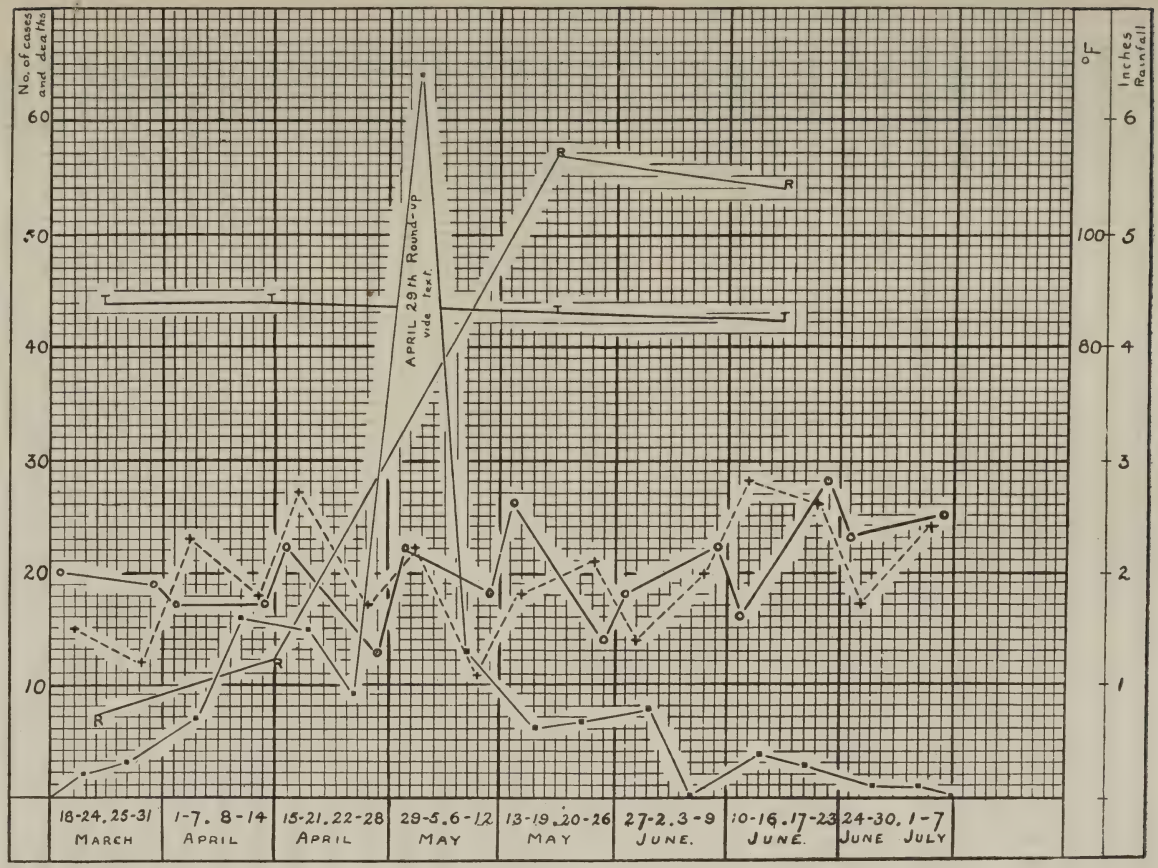
A chart is appended to show the progress of the epidemic from week to week. A somewhat erroneous impression is gained from an inspection of the chart, however, since the peak of the outbreak would appear to have occurred in the weekly period April 29th—May 5th. The large excess of cases occurring during this and the following weekly period resulted from legislation being passed on April 28th, which allowed the Health Authorities to round up nearly four hundred suspected cases and contacts with cases isolated from certain areas.

Briefly, a steady weekly increase in the number of cases reported occurred from the first case on March 18th, attaining a maximum during the weekly period April 29th—May 5th, and then steadily decreasing until only one case occurred during the weekly period June 24th—30th, this case being a second attack in a European infected experimentally in order to confirm the mode of transmission of infection.

(g) Meteorological

A daily maximum temperature of over 98.8°F. is stated to exert an unfavourable effect on lice. Conclusions based upon observations

CHART I. ILLUSTRATING THE PROGRESS OF THE OUTBREAK OF RELAPSING FEVER IN ACCRA WEEK BY WEEK.



•——• No. of cases of Relapsing Fever occurring in weekly periods.
o——o No. of deaths from all causes in Accra in weekly periods in 1923.....TOTAL 320.
+----+ " " " " " " " " " " " " 1922.....TOTAL 313.
T—— Average Shade Maximum Temperature.
R—— Rainfall in inches for the month.

carried out over a period of three months showed that the small variations in atmospheric temperature and relative humidity had little or no influence on the degree of infestation of the Northern Territory tribes normally found infested with lice.

The outbreak commenced towards the end of the dry season during March, when the average maximum shade temperature was 88°F. and the relative humidity 67.

The greatest number of cases occurred at the end of April and at the commencement of May, during which times the rainy season had been in progress for a short time, and the average maximum shade temperature and relative humidity for April and May respectively being 88°F. and 86·5°F. and 67·1 and 76·9. The epidemic virtually came to an end early in July during the continuation of a rather more than normally wet season. The slight fall in temperature and the decided increase in rainfall between March and July appeared to have little influence on the course of the epidemic or upon the severity of individual cases.

(*h*) *Morphological Characters of the Spironemata*

The spironema found in the blood of cases met with in the present epidemic differs in no way from the descriptions given of the spironemata causing relapsing fevers in other parts of the world. Two hundred spironemata taken as they came—twenty-five in eight blood films from separate cases—were drawn with the help of the camera lucida and measured by the compass method, Macfie and York (1917). The shortest spironema found measured 10μ and the longest 44μ, the average length being 21·9μ. The commonest lengths of the spironemata were 18μ to 23μ, and the average thickness of the spironemata was 0·3μ. The pleomorphism noted by the French observers, J. Kerrest, A. Gambier et A. Bouron (1922), in the Soudan epidemic of relapsing fever has also been noticed by us, but it appears to us to be merely a passing phase; in blood films obtained from the same case on consecutive days, we have found few, if any, irregular forms on the first day, while on the second day ring and figure-of-eight forms have been numerous and did not require to be searched for. Breinl (1908) states with regard to *Sp. duttoni*, that coiled and complicated skein-like forms are most numerous in the blood of the internal organs just before the crisis sets in. Balfour and Bousfield (1911) have described and

figured these irregular forms of spironemata in relapsing fever at Khartoum. With the exceptions of ring, figure-of-eight and partially coiled forms, the shape of the organism did not appear to undergo any change in patients from day to day and, although the majority of the films examined were air-dried before being treated with Ruge's fluid, the exposure of blood films to hot air, to the vapours of formalin, to osmic acid, or to chloroform, appeared to have no influence on the shape of the organism.

The spironemata found in emulsions of crushed lice appeared to be shorter and more delicate than those seen in blood films, they also stained less deeply with gentian violet.

The number of organisms found in thick blood smears varied from over forty per field observed in a case which resembled in many respects a typical case of lobar pneumonia to as few as two over the greater part of the slide.

It would have been anticipated that spironemata would have been more numerous in severe cases of the disease and in first attacks than in relapses, but this was not invariably the case, although as a general rule they were less easy to find in relapsing cases and in fact were rarely found in what appeared to be a relapse after injection of a substerilising dose of Novarsenobillon. In one case spironemata were found by Dr. Mary Magill (who kindly assisted to examine a group of nearly two hundred films prepared from contacts and suspected cases) in a blood film of a contact who was not suffering from pyrexia at the time nor for the forty-eight hours intervening between his blood being taken and his treatment with Novarsenobillon. This case showed no signs or symptoms of illness and was discharged fourteen days subsequent to his receiving an intravenous injection of 0·3 gm. of Novarsenobillon, not having shown any signs of sickness. This blood film was one amongst twenty-five other films of contacts, all of whom appeared and were healthy; thus the possibility of the slides having become mixed could be excluded.

It is a remarkable fact that a careful search through blood films taken from some patients who appeared to be suffering from typical attacks of spirillar fever, who were stricken at the same time as their comrades, and who reacted to intravenous medication in exactly the same way as their fellow patients, failed to show the presence of infecting organisms.

In this connection it is noteworthy that in the severely collapsed cases with subnormal temperatures spironemata were not discovered in thick films until reaction had set in and the temperature mounted to 100°F. or more. Owing to the system adopted of taking the temperatures of all contacts and suspects and of carrying out a routine blood examination of every person segregated, whether he suffered from pyrexia or not, conclusive evidence was obtained as to the absence of the organism in the blood in the apyrexial state with the sole exception of the case described above. Spironemata were not found in the specimens of sputa and urine obtained from relapsing fever patients.

ANIMAL EXPERIMENTS

The following animals were inoculated with blood obtained from cases of relapsing fever at the Colonial Hospital or at the Contagious Diseases Hospital, Labadi:—White rats, black rats, *Cricetomys gambianus*, guinea-pigs, monkeys and one rabbit. The quantity of blood inoculated varied usually from 0·5 ccm. to 2 ccm., citrated blood being employed in all but one of the experiments. The rabbit and guinea-pigs proved refractory, no spironemata being at any time found in their blood, which was examined daily for a fortnight after inoculation. Eight white rats were inoculated at different times with infected blood, but in only one of them were spironemata seen; this rat was given 2 ccm. of blood from a human case on the 21st March and on the following day, twenty-six hours after the inoculation, two spironemata were found in a thin film of its blood; on no other occasion in this rat were spironemata found, although the blood was examined daily for a fortnight. That the blood employed in the cases of two of these white rats inoculated on the 28th March was infective was proved by the inoculator unwittingly infecting himself and developing relapsing fever, the first symptoms of which appeared on the 4th April—seven days after infection occurred.

Three monkeys were inoculated. Monkey No. 1 received a few drops of serum only, as the blood, obtained from the first case diagnosed, was carelessly allowed to clot in the syringe; this monkey never showed any symptoms and spironemata were never detected in its blood, which was examined daily for a fortnight after inoculation.

Monkey No. 2—a small baboon—was given 2 ccm. of citrated blood containing spironemata on the 21st March. On the 23rd it was not so lively as usual; on the 24th it had a temperature of 102° F. and spironemata were numerous in its blood; they were less on the 25th, and had disappeared completely on the 26th. From this day onwards to the 6th April, when the daily examination of the blood was discontinued, no spironemata were found. This monkey, which has been under close observation for three months, has never shown symptoms of a relapse.

Monkey No. 3—a sooty mangabey—was inoculated with about 2 ccm. of blood containing spironemata on the 23rd April. It appeared rather subdued on the 25th, but otherwise showed no symptoms of being ill, and its temperature was only 100° F.; on the 26th spironemata were numerous in its blood, but had disappeared on the 27th, and after this date no spironemata were found in its blood. This monkey has also been under close observation for nearly three months and has shown no signs of relapse.

Three black rats (*M. rattus*) were inoculated. Two received about 1·5 ccm. of citrated blood on the 28th March; this was the same sample of blood which failed to infect two white rats but proved infective in the case of the inoculator. Neither of these black rats showed spironemata in its blood, which was examined daily for twelve days following the inoculation. A third black rat was given 4 ccm. of citrated blood from a human case on the 2nd July. Spironemata were fairly numerous in its blood on the 4th, but were absent on the 5th, and have never been found since that date.

Two *Cricetomys gambianus* Waterhouse were given large doses (4 ccm.) of citrated blood which contained spironemata on the same occasion as the black rat last mentioned. On the 4th July spironemata were found in the blood of one of them; on the 5th both showed spironemata in large numbers in thick blood films; on the 6th the blood of the first rat which showed spironemata on the 4th July was free from them, while that of the other showed them in large numbers; subinoculations were made from each of these rats into another rat of the same species on this date. Both subinoculated rats showed spironemata, but at different dates after inoculation; the rat receiving blood containing spironemata showed them in its blood on the third day, the rat receiving blood which was apparently

free from spironemata on the eighth day. The original two rats relapsed, one after its blood had been negative for four days, the other after its blood had been negative for seven days.

A white rat was subinoculated with blood from Monkey No. 3 when it contained numerous spironemata on the 26th April, with a view to finding if passage through a monkey exalted the virulence of the strain for white rats. The blood of this rat never showed spironemata on any occasion, though examined daily for a fortnight after inoculation.

The results obtained from these inoculations appear to correspond closely with the inoculation experiments conducted by Gambier (1923) at Bamako. Gambier found monkeys to be readily infected with spironemata, white mice to be infected with difficulty, and rabbits and guinea-pigs to be refractory.

Cricetomys gambianus—the pouched rat—showed itself to be much more susceptible to infection with spironemata than any of the other animals employed; it was the only animal which appeared to relapse. It should be possible to convey the strain to Europe by means of a series of these rats provided they can be got to survive the rigours of a northern climate.

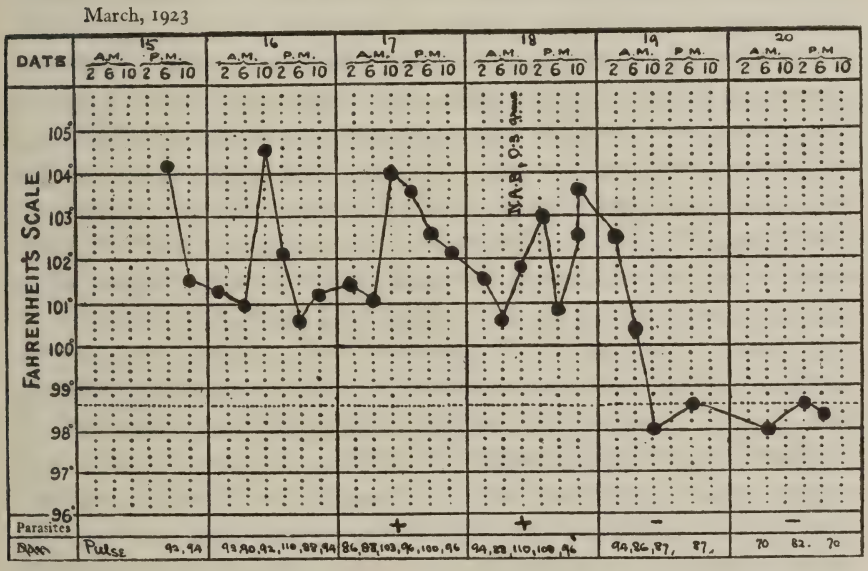
CLINICAL MANIFESTATIONS

The first case recorded was I. A., an Italian contractor, aged 26. He was admitted to hospital on 15th March, 1923, with pyrexia, headache and severe prostration. He had been resident in the Gold Coast Colony two years, engaged in contracting work, and had enjoyed good health. He was taken ill on March 14th. His blood, on admission, was found free from parasites and pigment. It was examined again on March 17th, when it was found to contain numerous spironemata. On March 18th he was given 0·3 gm. Novarsenobillon intravenously. The following day, being the sixth day of the disease, the blood was found free from spironemata, and the temperature dropped to normal, where it remained until the patient's discharge from hospital on March 24th. There was no relapse, and six weeks later I. A. sailed for Italy, apparently in perfect health. The other symptoms in the case were icterus of the seclerae, severe pains, especially in the thighs, vomiting, enlargement

of the spleen, and a severe nephritis. The urine contained albumen and a heavy deposit of granular casts; all this cleared up by March 23rd.

The only other European case was accidentally infected on two occasions. His illness ran a similar course, excepting that jaundice and nephritis did not occur. In this case the rheumatic pains, especially in the thighs, were excruciating. The second attack was quite similar to the first, and occurred after an apyrexial period of three months. It was much milder in character. Both attacks were treated with the intravenous injections of 0.3 gm. Novarsenobillon, and there were no relapses.

I. A., Italian, Male, Age 26. Relapsing Fever. Admitted 15th March.



The clinical manifestation in natives presented a much more varied picture.

Owing to the necessity for the employment of one and sometimes two interpreters in the case of Northern Territory patients, it was far from being an easy task to obtain reliable information regarding symptoms.

The incubation period was not definitely established, but appeared to vary from seven days in the case of an accidental infection resulting from spironemata in a drop of blood from a

patient, entering the system by way of a bruised nail bed, to twelve days in the case of another volunteer who allowed himself to be bitten by infected lice, and who is thought to have scratched a louse into his skin. In the case of two other volunteers, infection took place within eight or nine days of their receiving an emulsion of crushed lice rubbed into the scarified skin.

Prodromal symptoms were rare. The onset of the disease appeared to be sudden, and was often accompanied by a distinct rigor. Frontal headache became so marked in some cases as to warrant description by the patient as a pain like 'hammering on the temples.'

Some of the patients complained of severe pains in the cervical and lumbar regions, in thighs, shins and wrists. Prolonged attacks of shivering, rendering the taking of temperatures quite impossible, and very profuse sweating, were noticed in certain cases. Vomiting occurred in all but the mild cases, and persisted in some for two to three days after the fall in temperature, whether preceded by intravenous medication or not. The vomiting was at times bilious, but usually occurred after a drink of Akasa—a kind of pap—or after taking other food. The tongue was coated with white fur, and anorexia was marked during the course of the disease but gave place to a ravenous appetite in the majority of those who received an intravenous injection of Novarsenobillon. Thirst was severe, particularly, as would be expected, in those cases which suffered from frequent attacks of vomiting. The patients complained of giddiness when they attempted to stand; in some the gait was staggering, and others not only felt too giddy to stand in an erect posture, but collapsed when they even attempted to sit up. The asthenic condition persisted in some after convalescence had been established.

Both liver and spleen were enlarged in many cases, but as the patients for the most part came from malarial infected areas, it is possible that malaria was the cause of the splenomegaly, although diminution in size of the spleen was recorded during convalescence (quinine not being administered). Jaundice was present in remarkably few cases. In one series of one hundred and seventeen, only three patients suffered from jaundice.

The majority of the patients suffered from constipation, but a

certain number, particularly those in whom the temperature had fallen by crisis, suffered from diarrhoea.

The urine showed albuminuria during the pyrexial stages of the disease, but was not remarkable for any peculiarities. Oedema of face and hands, suggesting a nephritis, was seen in a small minority of cases.

Cough and a small degree of bronchitis were present in many of the cases, and in one pulmonary signs were so marked as to lead to a provisional diagnosis of pneumonia being made. Except in this case the pulse-respiration ratio was normal.

Mental symptoms were observed in a certain number of cases, and varied from a slight vacuity of mind and loss of memory to profound mental dulness and, in a small number, to active delirium and a comatose condition.

The pulse rates recorded were for the most part consistent with the height of the temperature in the pyrexial period, although in three cases out of a series of one hundred and seventeen in whom convalescence was prolonged owing to cardiac dilation, the rate remained unduly rapid for two weeks or more after the fall of temperature to normal. In the collapsed cases the pulse was of poor volume, thin and thready and often uncountable.

A somewhat unusual series of facts were noted in connection with the temperature recorded. It might have been assumed with all fairness that the severity of other signs and symptoms in a case of spirillar fever was proportionate to the height of the temperature. This was by no means the case. In some cases where the temperature rose to 104° F. or higher the symptoms were by no means severe, and recovery rapidly took place after suitable treatment. In other cases where the temperature did not rise much higher than 100° F. other signs and symptoms were grave. In the single case under the care of one of us (P.S.S-C.) which ended fatally the temperature on admission was 99° F. A thick blood smear was taken and large numbers of spironemata were observed. The patient was given an intravenous injection of 0·6 gm. Novarsenobillon and then put to bed. His temperature rose to 101° F. by 6 p.m. the same evening. On the following morning his temperature had fallen to 99° F., but his condition was grave and he could not be persuaded to take any fluid nourishment. A blood smear was negative to spironemata.

He vomited bilious-looking material twice during the day, and by 6 p.m. his temperature was 99·4° F. When seen on the following morning at 6-30 a.m.—probably the fourth day of his illness—he was found to be comatose. The thermometer did not register any temperature and his radial pulse was so small in volume and rapid in frequency as to be almost imperceptible and quite uncountable. A blood smear was negative to spironemata. Efforts were made to combat the condition by raising the end of his bed, by applying hot blankets, hot water bottles and by administering intravenous and subcutaneous salines and brandy. By 10-30 a.m. his temperature had risen to 97·2° F. and his pulse, though rapid (120), was of good volume and tension. Respirations which had been of the Cheyne-Stokes variety at 6-30 a.m. were now comparatively normal although the breathing was stertorous. By 2-30 p.m. the patient's temperature had risen to 101° F. His pulse was of good volume and tension and about 120. Breathing had become markedly stertorous and respirations numbered 36 to the minute. A blood smear was taken but no organisms were found. The patient died at 4 p.m. on the same day, having remained unconscious for over 48 hours. As far as could be gathered, the patient had been ill for two days prior to his admission to hospital, thus death took place within five days of the initial symptoms. The above case has been given at some length in order to show that the height of the temperature had little relation to the severity of an attack. It is to be noted that the blood smears taken on the second and third days of the illness prior to the patient receiving Novarsenobillon showed a very heavy infection of spironemata.

Another type of temperature was seen in the case of a female subject of good physique and aged 20. The temperature recorded on the first four days of her illness was 100° F. or less. A blood smear taken on the first day proved on examination to show a moderate number of spironemata. On the second day—the patient remaining without treatment—the number of organisms in a thick film was very small, and on the third day none were found. By the fifth day the temperature had fallen to 97·4° F., and it remained low for ten days. On the tenth day following the initial fall to below normal, the temperature rose to 100° F. On the following morning at 6-30 a.m. the temperature was 104·4° F. Spironemata were not

found in the blood until the eleventh day following the original commencement of the apyrexial period after the first attack. Owing to the obvious suffering of the patient and to her serious condition, one of us (P.S.S-C.) did not feel justified in withholding Novarsenobillon any longer and gave 0·6 gm. intravenously at 11 a.m. By 6-30 p.m. the temperature had fallen to 103° F. On the following morning the temperature still stood at 100° F. but fell to 99° F. the same evening and to 97° F. by the next morning. Spironemata were found in moderate numbers at the height of the relapse but not subsequent to the treatment with Novarsenobillon.

In a second patient—an adult male aged 44—untreated until the first relapse, the temperature on admission on the third day of his illness was 103.2° F. Spironemata were present in moderate numbers in a thick blood smear. The patient received no treatment other than a cold sponging, and his temperature fell the day after his admission to 97° F., remained normal or subnormal for two days and then rose to 101° F. During the apyrexial period, organisms were absent from blood smears, but were present on the day of the relapse. The patient appeared to be suffering considerably during the relapse, and it was not considered fair to him to withhold specific treatment any longer. The temperature fell to normal and spironemata disappeared from blood smears within twenty-four hours of the patient being injected with 0·6 gm. Novarsenobillon and no further relapse occurred.

Particulars of a fourth case are worthy of record since the patient appeared to be suffering from pneumonia on admission. The patient was admitted to hospital on the second day of the disease. His temperature, pulse and respiration at 2-45 p.m. on the day of admission were respectively 103·6° F., 100, and 40. Although a well nourished male of 25 he was too weak to move hand or foot and was delirious. Bronchitic râles were heard over both sides of his chest and signs of early pneumonic consolidation were heard over the left lower lobe. A blood smear showed the presence of spironemata in large numbers. The patient was very jaundiced. He was given 0·6 gm. of Novarsenobillon intravenously and his temperature, which rose to 104° F. by 6 p.m. the same evening, fell to 100° F. on the following day and then to 97° F. on the morning of the third day after his admission. Subsequently, the temperature rose again on

the evening of the third day following admission to 99·8° F. and to 100° F. on the morning of the fourth day, but fell to normal on the same day. From thence onwards the temperature went to a few points above normal for the next fourteen days and then steadied down to subnormal. The pneumonic signs cleared up without any signs of resolution, but the patient suffered from bronchitis for a fortnight following his admission to hospital. Spironemata were not found in the patient's blood subsequent to the treatment with Novarsenobillon.

When discussing the variations in temperature in cases of relapsing fever it would be unwise to take the four cases quoted above as typical examples. By far the majority of cases suffered from temperature varying from 100° F. to 105° F., though a small number showed spironemata in blood smears with a temperature of only 99° F. In most cases a fall of temperature to normal or subnormal took place within twelve hours of treatment with Novarsenobillon, although in some cases the temperature remained above normal though lower for two to three days and then fell. During the early days of the epidemic when only 0·3 gm. of Novarsenobillon was administered, a number of the patients relapsed after varying intervals. In a small minority of cases it was found necessary to give 0·6 gm. of the drug followed by 0·3 gm. after an interval of three days.

RELAPSE

The information regarding the occurrence of relapses is scanty for two reasons. Africans, and in this they resemble all races, do not take kindly to hospital treatment, and purely medical cases prefer to remain in their own homes rather than to enter hospital, however comfortless the former may be, and however much their chances of recovery may be so impaired. It follows naturally that if, owing to pressure having been brought to bear on them, they have been admitted to hospital for treatment, their one aim and object is to obtain their discharge therefrom as soon as possible. Consequently, when a case of relapsing fever was admitted to hospital, endeavours were made to sterilise the patient as regards the infecting organisms in his blood and to effect his cure with the least possible delay. By making his stay in hospital as short as was compatible

with his own well-being and with the safety of the general public, other cases occurring in the town were encouraged to seek medical attention as soon as they became infected, instead of remaining concealed from the health authorities and so helping to spread infection.

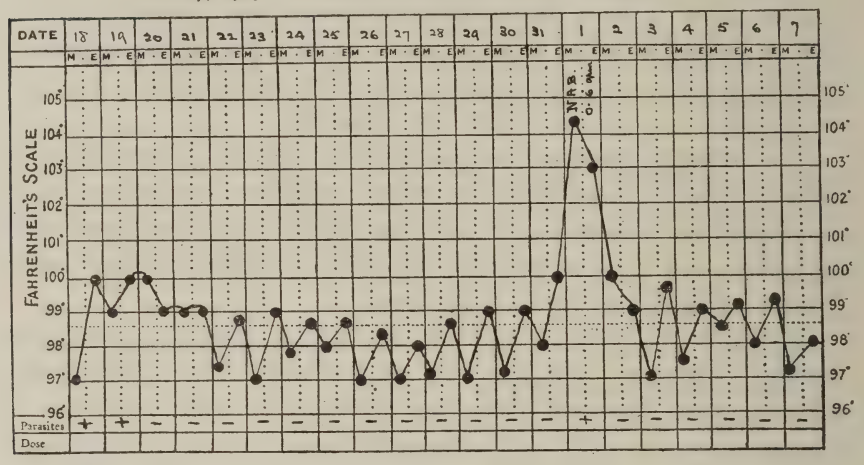
Secondly, it was not considered justifiable, except in a very small number of cases, to withhold treatment from a patient with a view to determining the approximate length of the apyrexial period between attacks, since by so doing, the well-being and possibly even the life of the patient was placed in jeopardy.

The relapses observed can be divided into two classes, one in which the patient had received no specific treatment, but only general symptomatic treatment as, for example, light diet, saline purge, cold sponging, and the second class in which specific treatment with Novarsenobillon had been administered.

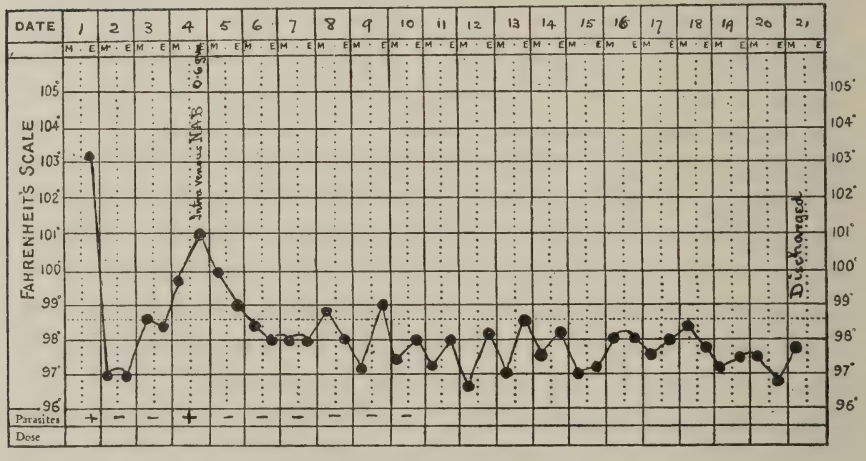
Examples of the first were afforded by the cases of a man and woman. The man was admitted on the third day of his illness suffering from the usual symptoms of relapsing fever and with a temperature of 103·2°F. Spironemata were present in moderate numbers in a thick blood film. The patient received symptomatic treatment only and his temperature fell to 97° F. on the day following admission and remained normal or subnormal for two days. On the third day following the crisis which terminated the first attack, the temperature rose to 101°F., and spironemata which had been absent from blood films during the apyrexial period of two days were again found in blood films. The condition of the patient did not justify the withholding of treatment further. The woman suffered severely from headache, pains in the back, legs and wrists, but her temperature during the first four days of her illness did not exceed 100° F., although a blood film taken on the first day showed a moderate infection with spironemata. The number of spironemata seen on the second day of the disease was smaller, while none were found on the third and fourth days. On the fifth day of the disease the temperature fell to 94·4° F. and remained low for ten days. On the tenth day following the initial fall to below normal the temperature rose to 100° F. and on the following morning at 6-30 a.m. to 104·4° F. Spironemata were found in moderate numbers, but the patient's condition was sufficiently serious to make further delay in the administration of specific intravenous medication quite unjustifiable.

Several examples of the second series of relapses, that is to say, relapses occurring in spite of treatment, occurred amongst the cases

ARMAH, Female, Age 20. Relapsing Fever. Admitted May 18th.



SEIDU SYIE, Male, Age 44. Relapsing Fever. Admitted June 1st.



treated in the Contagious Diseases Hospital. The following case is remarkable for its severity and for the unduly long period that elapsed between the patient's apparent cure and his relapse. The

patient, a well nourished young adult of 23, was admitted to hospital together with a large number of other cases, suspects and contacts, on 29th April, 1923, suffering from signs and symptoms of relapsing fever. On the 30th April his temperature was 105° F.

MUMUNI III, Male, Age 23. Relapsing Fever. Admitted May 2nd.

May, 1923 First attack.

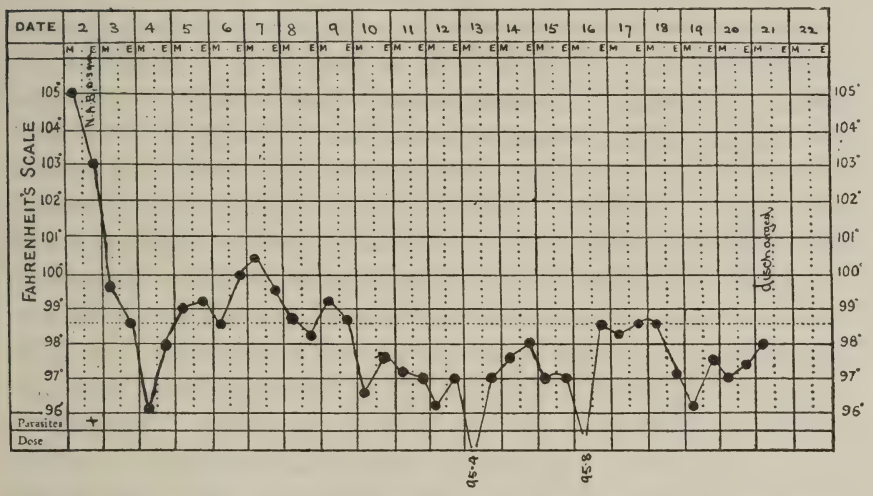
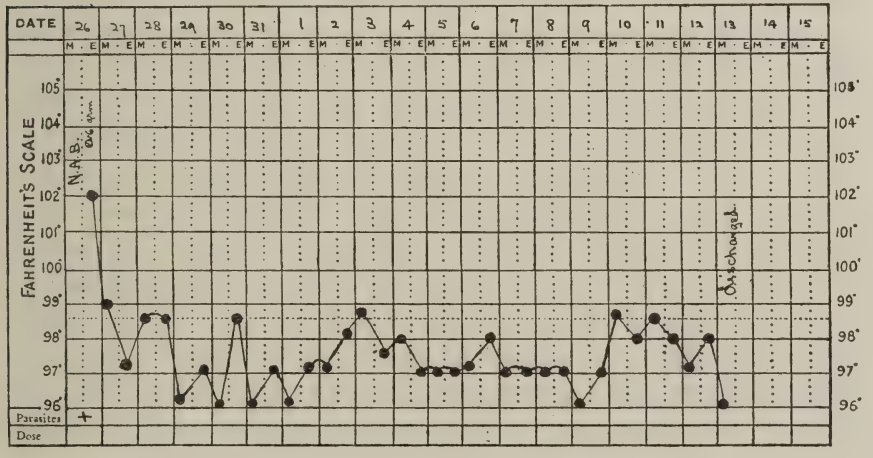


Chart to illustrate occurrence of relapse after an unusually prolonged period of apyrexia possibly resulting from the administration of an inadequate dose of Novarsenobillon during the first attack.

MUMUNI III, Male, Age 23. Relapsing Fever. Admitted May 26th.

Relapse.

May, 1923 June



On the following morning his temperature was 102·6° F. but a blood film failed to show the presence of spironemata. His temperature on the following day was 105° F., spironemata were present in a blood film, and he was given 0·3 gm. Novarsenobillon intravenously. By the evening of the same day the patient's temperature had fallen to 103° F. The next morning the temperature had still further fallen to 99·6° F., and by the evening to 98·4° F. For the 3rd to the 9th of May inclusive the temperatures were 96°, 99°, 98·4°, 98·6°, 99·2° F., but no spironemata could be found in blood films taken during this period. Thereafter the temperature remained normal or subnormal until the 21st of May when the patient was discharged. Three days later on the 24th of May (fourteen days after the patient's temperature had fallen to normal) the patient complained of anorexia, diarrhoea and frontal headache. He was seen on the 26th of May and found to have a temperature of 100°F., and spironemata were observed in his blood film. On this occasion he was given 0·6 gm. of Novarsenobillon. His temperature rose to 102° F. by 6 p.m. the same night, but had fallen to 99° F. at 6 a.m. on the following morning. Organisms, however, were still visible in blood films. The temperature fell to 97·4° F. the same evening and subsequently remained normal or subnormal until the 13th June when he was discharged, his temperature not having been raised for sixteen days. The interesting point about this case is that the original pyrexial period lasted for about eleven days, followed by an apyrexial period of about fourteen days, when a relapse occurred. The pyrexial period during the relapse lasted for four days and was followed by a period of apyrexia for sixteen days. The explanation in this case is that the 0·3 gm. of Novarsenobillon administered was too small a dose to sterilise, but that it probably had the effect of retarding the relapse which took place fourteen days after the patient's temperature had been normal or subnormal. During the relapse, spironemata were present in large numbers in blood films until the third day of the disease, when a 0·6 gm. dose Novarsenobillon was given. Organisms were present in small numbers on the morning following the injection, but disappeared from blood films taken thereafter. Several similar cases occurred in which an initial dose of 0·3 gm. of Novarsenobillon appeared to effect a cure, but in which it ultimately proved to be insufficient in preventing the occurrence of a relapse.

A few patients relapsed even after receiving 0·6 gm. of the drug, but for routine work, dealing with a large group of persons, this dose appeared to be satisfactory, followed by 0·3 gm. or 0·6 gm. in the small number of cases failing to react to the initial dose, or showing signs of relapse.

Briefly, in untreated cases relapses occurred with an apyrexial period varying from two to ten days, while in treated or partially treated cases the apyrexial period varied from two to fourteen days. As a rule, but not invariably, the relapse was less severe both in signs and symptoms and also in duration than the initial attack. Out of the hundred and seventeen cases that came under the care of one of us (P. S. S-C.) twenty-three, or 19 per cent., relapsed on one occasion, and eight, or 6·8 per cent., relapsed a second time. Thus the total number of relapses in the one hundred and seventeen patients was thirty-one, or 26·5 per cent., of all the patients.

The following table shows the results:—

Table IV.

	Blood film		Unknown	Total
	Positive	Negative		
First Relapse	11	7	5	23
Second Relapse	3	5	...	8
Totals	14	12	5	31

In cases where the organisms could not be found in blood films, the diagnosis of relapse was based upon rise of temperature and the recurrence of the signs and symptoms of the original attack.

IMMUNITY

Second Attacks. Immunity is said to be of short duration in relapsing fever. It would be unfair to draw any such conclusions from the epidemic under review, since so little time has elapsed since the occurrence of the cases described above. One undoubted second attack occurred, however. The patient, a European, originally became accidentally infected with relapsing fever while injecting

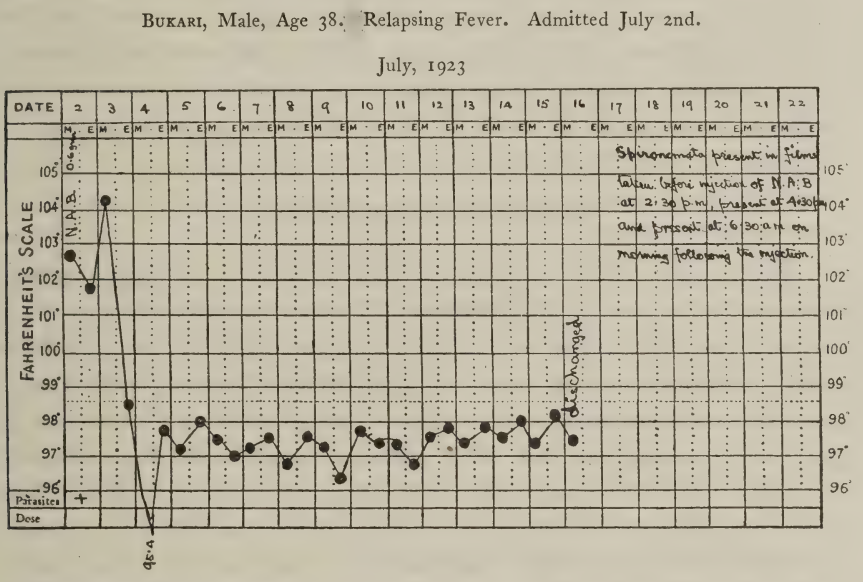
infected blood into a rat. Seven days later he developed the signs and symptoms of the disease. At first no spironemata were seen in his blood, but after two days of moderately severe pyrexia the organism was found to be present in small numbers. The patient was treated with 0·3 gm. Novarsenobillon intravenously, and rapidly recovered. This first attack occurred in the beginning of April. At the end of June the same individual contracted a severe attack of the disease as the result of feeding infected lice on his forearms—two escaped and are thought to have been scratched into his skin. The second attack was rather less severe than the first, but reacted to treatment with Novarsenobillon as rapidly as had been the case in the first attack. If the patient acquired any immunity from the first attack, and this is probable, since he subsequently carried out a series of experiments, feeding on his forearms lice from relapsing fever patients, the immunity was of decidedly short duration, in fact less than eleven weeks.

TREATMENT

Apart from intravenous medication with Novarsenobillon given intravenously in 10 c.c. of warm, sterile, distilled water in doses varying from 0·3 gm. in the early cases to 0·6 and 0·9 or 1·2 gm. (divided into two to three doses) in the later cases, the treatment administered to patients was symptomatic. Patients were washed, shaved and disinfested on admission, and put to bed with a sleeping mat and two warm blankets. They received their injection of Novarsenobillon as far as possible on a fasting stomach. If they wished, they were given two biscuits and a drink of milk a short time after the injection. Cold sponging was resorted to where the temperature was 103° F. or higher, but otherwise, apart from being given as much water in small quantities as they wished, the patients were left to sleep quietly until the following morning, care being taken to avoid chills in cases where the temperature fell by crisis. The following morning a saline purge was administered, and if conditions were satisfactory and no vomiting was present a light diet was given. It was noticed that the patients not only wanted to get up and resume their usual everyday life immediately after the fall of temperature had occurred, but that they wished to resume a normal diet at once. As a rule the attack left the patients somewhat weak,

and vomiting immediately occurred, if the patients were allowed to satisfy their ravenous hunger. In those patients who reacted well to the treatment and in whom no elevation of temperature occurred within the fourteen days subsequent to the fall of temperature to normal, attempts were made as far as possible to graduate both diet and exercise until, for some days before the patients were due for discharge, they had resumed a normal life.

In order to obviate the possibility of relapses, patients were kept in hospital until their temperatures had been normal for fourteen days. Blood smears were taken of all patients with temperatures



Typical moderately severe case showing fall of temperature by crisis 24 hours after administration of 0·6 gm. Novarsenobillon.

above 99° F., and even if spironemata were not found in the blood films and other conditions could be excluded, further treatment with Novarsenobillon was carried out. This step appeared to be justified in the light of the subsequent histories of such cases. Among the one hundred and seventeen patients admitted to the Contagious Diseases Hospital, eighteen had already received an intravenous injection of 0·6 gm. Novarsenobillon. Injections—three of which were given intramuscularly owing to the inability to discover a vein of adequate size—to the number of one hundred and

thirty were given by one of us (P. S. S-C.). Of the total number of injections one hundred were of 0·3 gm. and forty-eight of 0·6 gm. Patients who received but one injection numbered one hundred and seventeen, those who received two numbered twenty-three, while eight patients had to receive a third injection. Generally speaking, apart from a little vomiting for two or three days after the injection (this vomiting occurred in untreated cases and consequently may have borne no relation to the intravenous medication) no ill-effects were experienced from the use of Novarsenobillon. One death occurred in a series of one hundred and seventeen cases that came under the notice of one of us (P. S. S-C.), but the patient was in a serious condition prior to the injection, and when death occurred on the third day following the injection, it could be attributed with fairness to the disease. West Africans appear to tolerate organic arsenical preparations exceedingly well.

PREVENTIVE MEASURES

The control of the outbreak of relapsing fever in Accra during the earlier stages was hampered by two considerations, viz., ignorance of the vector and mode of transmission of the particular strain of organism and lack of legal powers to deal with cases, suspects and contacts. The vector being unknown, lice, ticks, bed bugs, mosquitoes and biting flies were all treated as suspect. Samples of all these were collected either, as in the case of the first two from patients known to be suffering from the disease or, as in the case of the remainder, from bedding, mats and other articles in infected premises, and were taken to the Medical Research Institute.

Measures were taken against all possible vectors, and with this end in view attention was concentrated on premises situated in congested areas of the town occupied by Hausas and members of Northern Territory tribes, whose habits in regard to overcrowding and a marked aversion from cleanliness, adequate lighting and ventilation were well known. Careful house-to-house visits were made in those areas and throughout the town, as many as 46,013 being carried out during March, April, May and June, one of us (P. S. S-C.) being responsible for 1,006. During these inspections personal, domestic and general cleanliness was preached, all old

sacking, lousy bedding and other refuse being removed from compounds. Moreover, efforts were made to see that all houses were provided with adequate lighting and ventilation. During these visits a practice was made of urging any person who appeared to be ill and suffering from fever to go for treatment to the Native Hospital.

The procedure adopted early in the epidemic when a case of relapsing fever was reported was for the patient to be admitted into the Native Hospital, for his quarters to be disinfected and disinfested and for a watch to be kept on all contacts, any of whom showing signs of fever being urged to report to the Medical Officer at the Native Hospital.

By the fourth week of the outbreak it was evident that infection which appeared to have been introduced into Accra from bush villages was spreading to other parts of the town from houses already infected with relapsing fever. To obviate this tendency to spread, it was strongly urged that legislation should be passed, in order that the Health Authorities might round-up all persons suffering or suspected to be suffering from relapsing fever, together with contacts with such cases, for the purpose of segregating them and sterilising them as far as concerned the presence of spironemata in their blood. Legislation was not passed, however, until the 28th of April, by which time the disease had appeared in several parts of the town, though principally in Tudu and the Zongo. On the 29th of April, acting within the powers obtained through this legislation, it was possible to effect a round-up of cases, suspects and contacts on a much larger scale, so that within twenty-four hours of legislation being passed, over three hundred and sixty-three patients, suspects and contacts were removed from infected premises to the Contagious Diseases Hospital. Where necessary, one or two contacts were allowed to remain in such infected premises, to safeguard the property from thieves, to keep the premises clean and to care for any horses or other animals. Such persons were visited daily to exclude the possibility of their having contracted the disease.

The health of the person permitting, a routine was adopted at the Contagious Diseases Hospital in almost every case. On admission all male cases, suspects and contacts had their heads,

armpits, beards and pubic hair shaved, while in the case of females the hair was close clipped and shaved from their armpits and pubes under the supervision of a female sanitary inspector. Subsequently the shaving was followed, where physical conditions permitted, by a sea bath and by a wash-down where a sea bath was inadvisable. All clothing was shed into barrels containing a 5 per cent. solution of Izal prior to the bath being taken, and after the bath a warm blanket, sleeping mat, and cup and plates were issued to everyone. Temperatures and blood smears were then taken and recorded and the groups dealt with were allotted accommodation in three classes of huts, according as to whether they were thought to be suffering from relapsing fever or were merely suspects, or contacts with cases and suspects. Special diet, as for example, milk, tea, broths, etc., was given to patients, whilst the remainder received two meals per day. Hot Akasa was given in the early morning as soon as temperatures had been taken, and was followed at mid-day by a large meal of rice, plantain, fula or other foodstuffs purchased in the markets. As far as possible the tastes and wishes of patients and contacts were consulted with regard to the variety of food supplied. Contacts were detained for fourteen days, during which time they were given a certain amount of work to do in the way of scrubbing out huts, keeping the segregation compounds clean, helping with the chopping-up of firewood, with the preparation of food and drawing of water. They enjoyed sea baths daily, arrangements being made for the opposite sexes to bathe at different times. At the end of the quarantine period, if their temperatures which were taken morning and evening had remained normal, the contacts were again submitted to a thorough shaving, and were given a bath, and then had their disinfected and disinfested clothing returned to them and thereafter were discharged. Contacts or suspects who developed raised temperatures, or in whom blood films proved to be positive as regards the presence of spironemata, were immediately transferred to the huts reserved for patients. After receiving appropriate medication, patients were kept in hospital until they had been free from pyrexia for fourteen days—blood films being taken daily while temperatures were raised.

It is noteworthy that the following method of disinfecting and disinfesting clothes and blankets appeared to give the best results.

The articles to be disinfested were first soaked for forty-eight hours in barrels containing 5 per cent. solution of Izal. They were then washed and placed in the sun during the middle of the day on sheets of corrugated iron. This resulted in most efficient disinfestation, for the heat generated was at least 150° F. Neither lice nor eggs capable of hatching survived this treatment. Purses and amulets, the latter carried in great numbers by Hausas and Northern Territory tribesmen, required special treatment.

In order to minimise the risk of infection being carried from the hospital to Accra, the auxiliary staff of the hospital were persuaded to stay in special quarters reserved for them in the grounds of the hospital, and all the staff, including one of us (P. S. S-C.), took further precautions by frequent baths and by shaving the hair from axillae and pubes. A police guard was maintained at the hospital during the period of the outbreak, and the Non-Commissioned Officer in charge is to be congratulated in not losing a single patient or contact.

CONCLUSIONS

1. This first recorded outbreak of relapsing fever in British West Africa is due to a spironema conveyed by lice.

2. As regards inoculation experiments, monkeys, black and white rats become infected with the strain, but do not relapse; guinea-pigs and rabbits are refractory, whilst the pouched rat becomes infected and relapses.

3. The vectors of the organism in the present epidemic and the inoculation experiments suggest that the parasite is not the *Sp. duttoni*, but corresponds more closely to *Sp. recurrentis* (vel *obermeieri*), or a related strain.

4. Novarsenobillon is a specific in the treatment of the disease.

5. Immunity does not appear to be lasting or complete in cases treated with Novarsenobillon.

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CARBON TETRACHLORIDE IN FILARIASIS

BY
S. ADLER

(*Sir Alfred Lewis Jones Research Laboratory*)

(*Received for publication* 23 *August*, 1923)

In view of the successful treatment of intestinal nematodes with carbon tetrachloride administered orally, it was decided to try the effect of the drug administered intravenously and intramuscularly on filariasis.

Prior to treating infected human beings, a series of experiments were carried out on healthy dogs. It was found that dogs could stand relatively large amounts of the pure drug administered intramuscularly or intraperitoneally, and that older animals tolerated the drug better than young animals; the minimal lethal dose was found to be 0·25 ccs. (*i.e.*, 0·39 gms.) per kilo body weight in animals weighing less than 1 kilo, whereas animals weighing over 4 kilos showed symptoms from which they recovered after a dose of 0·6 ccs. (*i.e.*, 0·94 gms.) per kilo body weight; these symptoms were drowsiness and refusal to take food for a few days after the injection.

Injected intravenously the drug caused rapid death due to embolism, but when mixed with two parts of ether by volume, embolism after intravenous injection was avoided.

An intramuscular injection of 0·5 ccs. of the drug into a healthy human being caused irritation at the site of injection, which was not severe and passed away in a few minutes; shortly after the injection the distinctive taste of the drug was felt in the mouth.

After these preliminary experiments the drug was tried on four adult patients each with a slight infection of *Filaria bancrofti*, as judged from the number of microfilaria in the circulating blood. In each case the number of microfilaria per c.c. was estimated by counting the number of microfilaria in 20 cmms. of blood at 9 p.m., before commencing treatment and at the end of treatment.

Case 1. A native aged 35, weight 144 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—50 per c.c.

25.6.23. Intramuscular injection of 0.5 c.c. carbon tetrachloride.

20.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 1.5 c.c. carbon tetrachloride.

30.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

Case 2. A native aged 32, weight 122 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—100 per c.c.

20.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 1.5 c.cs. carbon tetrachloride.

Case 3. A Native aged 25, weight 143 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—350 per c.c.

11.7.23. Intramuscular injection of 1.1 c.cs. of carbon tetrachloride.

16.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 1.5 c.cs. carbon tetrachloride.

30.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

Case 4. A native aged 24, weight 154 lbs.

Number of microfilariae in the blood at 9 p.m. before commencement of treatment—100 per c.c.

20.7.23. Intravenous injection of 1 c.c. carbon tetrachloride mixed with 2 c.cs. ether.

25.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

30.7.23. Intramuscular injection of 2 c.cs. carbon tetrachloride.

All the intramuscular injections were given into the buttock.

The patients were observed for one hour after each injection.

The intramuscular injection caused local pain, which passed away in from five to ten minutes. No marked analgesic effects were noticed after the local pain had disappeared. Injection of even 2 ccs. of the drug intramuscularly produced no anaesthesia.

Generally there was a slight diminution in the pulse rate up to four beats per minute following intramuscular injection.

All the cases noticed the taste of the drug shortly after injection.

Intravenous injection of the drug mixed with ether caused a severe attack of coughing, which commenced during the injection and lasted a few minutes; one case complained of a burning sensation in the mouth; all the cases were sleepy after the intravenous injection,

but whether the sleepiness was caused by the ether or the carbon tetrachloride it is impossible to say.

None of the cases showed albuminuria or other ill-effects either during or after the treatment, which was abandoned after the 30th July, 1923.

None of the cases showed any marked diminution of the microfilaria in the blood after the treatment, but it is impossible in the case of *Filaria bancrofti* to form an opinion of the effect of the drug on the adult worms.

The action of intravenous or intramuscular injections of carbon tetrachloride on adult filaria can only be tested in cases of Loa loa, but up to the present no suitable cases have been found in Freetown.

In view of the comparative safety with which the drug can be administered, both intravenously and intramuscularly, it is hoped that it will be tried on cases of Loa loa in localities where that disease is common.

I have to thank Dr. J. Y. Wood, of the W.A.M.S., for the opportunity of carrying out the above treatment, and Dr. P. A. Maplestone, from whose series of routine examinations for parasites the cases were selected.

YELLOW FEVER IN THE GOLD COAST: ITS ENDEMIC AND EPIDEMIC CHARACTER

BY

R. O. WHITE

WEST AFRICAN MEDICAL SERVICE

(*Received for publication* 24 *August*, 1923)

The recently reported cases of yellow fever in different parts of the Gold Coast* bring into prominence again the subject of its endemicity in West Africa, and suggest a consideration of the conditions which give rise to these periodic outbreaks.

That the West Coast of Africa has been an endemic centre no one disputes, but opinion is divided upon the question of the presence of yellow fever as an endemic disease to-day.

The Yellow Fever Commission (West Africa) appointed by the Colonial Office in 1913 considered the evidence submitted was in favour of the belief that West Africa is an endemic centre (1916). More recently, Guiteras (1921) and Hoffman (1921) expressed the opinion that this centre has already ceased to exist. This supports the view held by Ross, who suggests that the Stegomyia mosquito is not sufficiently numerous to permit the disease to maintain itself.

The investigations of the Rockefeller Foundation in 1920 failed to discover a single case, and we find that out of nearly half a million recorded illnesses treated in Nigeria during the years 1919-21, there is but a single diagnosis of yellow fever.

In spite of this formidable array of opinion and facts, it is not possible to ignore the significance of the existence of a disease which does not conform to prevalent types of fever and which is capable of being diagnosed as yellow fever.

The reports of the recent cases occurring among Europeans on the Gold Coast leave very little room for doubt as to the accuracy of the diagnosis. In all these were the black tarry or coffee grounds vomit, a marked diminution in the quantity of urine—amounting in

* A cable received at the Colonial Office on July 11, 1923, stated that 25 cases of yellow fever had occurred in the Gold Coast since November 1, 1922—18 in Europeans, all fatal, and 7 in natives, 2 fatal.

some cases to complete anuria—with albuminuria, associated with symptoms of extreme urgency followed rapidly by death. When post-mortem examinations were made, fatty degeneration changes were found in the liver and kidney. These features were common to all, but haemorrhage from mucous membranes, conjunctival injection, varying degrees of jaundice, Faget's sign and high fever are described. It is also noteworthy that, with one exception, the fatal termination occurred within a week of the onset of the illness.

In the face of such evidence, it must be admitted that yellow fever is a disease of West Africa. The next important point is—in what form does it exist?

An examination of the trade routes between the West Coast of Africa and other parts of the world fail to supply us with an external source of infection. In the 1911 outbreak at Bathurst it was thought that the occurrence of the disease at this port was connected with the visit of the s.s. 'Akassa.' Subsequent enquiries, however, failed to confirm this. In the epidemic under consideration, there is not the slightest ground for suspecting that the disease was introduced from without. It seems, then, that we must assume that yellow fever does exist in West Africa, in some latent form requiring special conditions for its development.

That special conditions are required may be gathered from the infrequency with which the disease occurs among Europeans in any one place, notwithstanding that the essentials for the maintenance and spread of the disease are always present—the virus, the carrier and the non-immune.

The explanation which used to be given for the comparative freedom from yellow fever among Europeans on the West Coast of Africa was, that the newcomer usually became the subject of a mild infection which conferred immunity for the remainder of his stay in the country. But this contention cannot be maintained, in view of the fact that victims of the disease have succumbed to it after periods of residence up to thirty years. It is necessary, therefore, to look for some other explanation.

When an endeavour is being made to discover the aetiological factor concerned in the outbreak of an infectious disease, it is helpful to be able to note the conditions under which it has died out in certain places.

Peterson's (1922) view of the spontaneous elimination of yellow fever in St. Thomas—which is based upon Carter's principle of 'the failure of the human host'—suggests that the virus gradually becomes attenuated until it reaches the point of extinction. In other words, the commerce between the mosquito and the unresponsive native eradicates the disease, not by the process of conferring general immunity, but by the death of the virus. The logical inference which may be drawn from this is, that the West Coast of Africa would cease to be a yellow fever area as soon as, or shortly after, less immune individuals were excluded from the country. The European is, of course, the obvious non-immune. Yet if we study the records of yellow fever epidemics, we shall find long intervals between outbreaks in any one place, and that they are traceable to a non-European source. This is fair presumptive evidence that the European is an accidental victim rather than an agent in maintaining the disease.

It may be urged that mild cases occur among Europeans which escape diagnosis. The mortality rate in the 1910-11 outbreaks (1913), which show six recoveries in forty cases, is against this; more especially when the reports of these recovered cases are scrutinised.

Of the six, three are included without any details of their illness, and as such, merely indicate people who were sick at the time.

Of the three remaining, two are so untypical of yellow fever, that it is safe to presume their inclusion was due to the prevalence of the disease rather than to the character of their physical signs and symptoms. The last case of this group appears to have responded, eventually, to energetic treatment with quinine. Albuminuria was absent throughout the illness.

In the present epidemic in the Gold Coast, the mortality among the Europeans attacked is 100 per cent. If we exclude the six doubtful cases referred to above, it would appear that yellow fever in West Africa is invariably fatal to Europeans. At any rate, it may be said it shows itself in a way which is not likely to be overlooked.

To recapitulate: The indigenous native, dissociated from the presence of newcomers, is incapable of maintaining the virus. The disease is not overlooked when it occurs among Europeans. The intervals which elapse between outbreaks among Europeans are proof

that the European is not responsible for the fact that yellow fever is an endemic disease in West Africa.

It is obvious then that some other section of the community keeps the virus alive in a ' larval ' state.

It has been said that previous recorded outbreaks are traceable to a native source. In the Reports of the 1910-11 epidemic (1913) it will be found that in every case when mention is made of living conditions, there is the association of close native proximity to the European attacked. Further it will be found that the native element is an imported one, either from the confines of the colony itself, or from a more remote part of West Africa.

Cases numbers 43 and 44 were Kroo-boys. 37 was a Yoruba lately come to Lagos, where he was taken ill. 29 and 38 also occurred in Kroo-boys. 28 was a Hausa. 19 and 20 were of the Mendi tribe living in Freetown at the time they were taken ill.

The foregoing comprise all these cases among natives where it is possible to identify their nationality. The remainder come under the, not very illuminating, description ' A native born and bred in West Africa.'

In the Gold Coast epidemic of this year, the cases at Saltpond were traceable to a Kroo-boy who died of yellow fever shortly after his arrival there. Later, eighteen miles away, Cape Coast was attacked, isolated cases subsequently occurring at Winnebal, Accra, Keta and Seccondee.

On the Gold Coast we find that West Africans who come to the Colony pursue one of the following callings:—Trading, soldiering, mining, as railway and road labourers, or manual work at the seaports. With the exception of the last-named class, it will be found that the principle of segregation is conformed with. The migratory trader, the Hausa, has his Zonga to go to, the soldier his barracks, while railway and road labourers (usually drawn from the Northern Territories) have carefully supervised camps.

The porter of the Gold Coast, the Kroo-boy, has no such provision made for him, and he is to be found, as a rule, domiciled in the compound of his employer, usually a European. He also has access to the native quarters of the town, and consorts with other Kroo boys who are employed by native merchants. There is here, then, a connecting link between the two classes which have been

excluded as not being responsible for maintaining the disease. The Kroo-boys also represent, numerically, a section of the community which must be taken into consideration, and they become suspects, partly because the process of elimination adopted here has left them unexonerated, and also for the reason that they are known to contract the disease and to have been responsible for outbreaks among Europeans.

The question now arises, how is it that these Kroo-boys are more susceptible to infection than the indigenous native?

It will be shown later that the immunity to yellow fever with which all West Africans are endowed is merely relative in degree and breaks down under certain conditions. It is suggested here that one such condition is change of environment.

Apparently the native's degree of immunity is sufficient so long as he remains in his own country, but becomes impaired when he goes to live in another part of West Africa. There is support for this assumption in the fact that West Africans do contract 'fever' when they leave one part of West Africa to take up residence in another. This has been recognised by Government Medical Officers, and the West Africans themselves are aware of it. An opportunity of observing this phenomenon occurs when a native official is transferred to a new station, or is on leave from a Colony of which he is not a native. That the 'fever' mentioned above is often due to malaria, there is very little doubt. An intensive and fatal case of this disease was seen at Accra in a Kroo-boy who had been resident in the Colony for six months. It is known that malaria does not attain to such severity in the adult indigenous population of West Africa. The influence of environment is, therefore, a factor which must be reckoned with when the subject of immunity is being considered.

From these premises it is easy to reconstruct the sequence of events. The Kroo-boy who migrates to some other part of West Africa automatically becomes more susceptible to yellow fever. He contracts the disease in a mild form, unrecognisable as such, and causing very little, if any, inconvenience. But an impetus has been given to the virus which, under favourable conditions, ultimately becomes so enhanced as to give rise to definite illness. The final stage is reached when living conditions make it possible for the

Kroo-boy to pass on this infection to a European. When this occurs the virus has attained to a very virulent degree of toxicity, which if unchecked by the wholesale destruction of the mosquito, will be capable, ultimately, of infecting—sometimes with fatal consequences—the indigenous and erstwhile unsusceptible native. This happened both at Saltpond and Cape Coast during the present epidemic, and is proof of what has already been said, that the West African's immunity is merely a relative one.

It is well known that any break in the chain of essentials which go to produce a yellow fever infection is sufficient to stop, or at least interrupt, the process. The lapse of time which takes place between observed epidemics in West Africa, seems to suggest that the chain is delicate in its construction and that the process of building up the virus sufficiently to produce recognisable effects is a long one. Segregation of Europeans—in as far as it obtains in West Africa—appears to have the effect of lengthening the process. It has certainly provided immunity for the segregated, for in no single instance has a case occurred among them. When it is remembered that in segregation areas native servants—often Kroo-boys and natives of the Northern Territories—live in close contact with the European, it would seem that the slightest precautions are sufficient to prevent infection. As Carter suggests in his statement of requirements for the maintenance of a yellow fever infection, the number of mosquitoes may fall short of what is necessary. It probably will be found also, that non-interference with the mosquitoes, overcrowding and lack of light and ventilation are necessary. Routine sanitary work probably intereferes from time to time with one or other of these subsidiary requirements, and has the effect of delaying the development of the virus. But sooner or later, it would seem, an area escapes over a period which permits it to become intensely infective, and an outbreak of yellow fever results.

The localised character of these outbreaks in a town is due to the well-marked domestic habits of the mosquito concerned. If we look at the spot maps accompanying the Reports of the 1910-11 epidemic, it is easy to see the human agency which carries the disease from one part of a town to another, over distances which leave intermediate areas unattacked.

It is, therefore, the infected rather than the infective element

which is responsible for the spread of the disease. If we can control the former and keep it from coming into close living contact with the unsegregated European, there is a reasonable prospect of preventing re-occurrences of these outbreaks. Efforts at controlling the other element have hitherto met with very little appreciable success. That temporary success is obtainable has been amply demonstrated during the present epidemic. Towns where the Stegomyia index is normally 80 per cent. have, after a week's intensive work, had this figure reduced to below 5 per cent. The means employed, other than fumigation of the area in which cases occurred, were the usual mosquito brigades under the supervision of European volunteers.

The effectiveness of this measure, when considered in the light of what has already been said, suggests the advisability of instituting a 'cleaning-up week' at least once a year, in every town where cases of yellow fever have been known to occur within the last twenty years. It should also be a matter of routine that when a case of yellow fever is reported in a Colony, every town with which the infected area is connected by road, rail, or sea, should immediately start energetic anti-stegomyia measures. This will prevent outbreaks elsewhere, for the reason that the number of mosquitoes remaining will not be able to maintain the disease. At any rate, the possibility of a secondary focus being established will be a very remote one. In the intervals between epidemics, Government Medical Officers and other Medical practitioners should be asked to observe carefully cases of fever which occur in West Africans who are strangers in the place, with a view to early diagnosis, thus ensuring prevention of the development and spread of the disease.

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MISCELLANEA*

KURLOFF BODIES IN FISH

In a specimen of *Diodon hystrex* examined at Freetown, Sierra Leone, Kurloff bodies were found in about 70 per cent. of the lymphocytes. As many as five Kurloff bodies were present in some cells.

S. ADLER and E. J. CLARKE.

TRICHONEMA TETRACANTHUM (MEHLIS, 1831, OF LOOSS, 1900)

This worm was found by us in June, 1923, in a donkey born and bred in the north of Ireland. This record is of interest, as the parasite has not been found since it was described by Looss in 1900. The fact that it had not been observed in Europe is used by Railliet (1923) as an argument that Looss' parasite is not identical with *Strongylus tetracanthus*, Mehlis, 1831.

J. W. S. MACFIE and WARRINGTON YORKE.

PIGS AND *ANKYLOSTOMIASIS* IN THE GOLD COAST

During January and February, 1922, forty-eight pigs were examined at the Accra slaughter-house for hookworms, but neither *Ancylostoma duodenale* nor *Necator americanus* were found, although 3,270 other small nematodes were collected, the majority of them being *Oesophagostomum dentatum* (Rud., 1803), and a few

* It is proposed to publish under this heading short records relating to Tropical Medicine and Parasitology.

Arduenna strongylina (Rud., 1819) and *Characostomum longe-mucronatum* (Molin, 1861). Subsequently specimens obtained from pigs at Cape Coast, at Kumasi, and at Sekondi (Dr. J. F. Corson) were examined, and in these also neither *A. duodenale* nor *N. americanus* was found. These results do not, therefore, support the view that pigs are an important factor in the dissemination of hookworm infections in the Gold Coast.

J. W. S. Macfie.

ONCHOCERCA ARMILLATA IN CATTLE IN THE GOLD COAST

Commes and Devanelle (1917) record that in Upper Senegal and Niger, *Onchocerca armillata*, Railliet and Henry, 1909, is a common parasite of cattle, and that they found it in one hundred and fifty-one animals out of one hundred and ninety-eight, that is in 76·3 per cent. It is also a common parasite of cattle in the Gold Coast, particularly of the hump-backed breed, as is shown by the fact that of forty animals examined at Accra during September and October, 1922, namely, sixteen hump-backed cattle and twenty-four of the straight-backed breed, fourteen, equal to 87·5 per cent., of the former, and seven, equal to 29·2 per cent., of the latter, were infected.

The situations in which the worms were found and the lesions (atheroma, calcification, cyst and nodule formation, etc.) associated with them were similar in the Gold Coast cases to those described by Commes and Devanelle, and need not be referred to in detail. Some of the nodules contained, in addition to a mass of fibrous material and portions of parent worm, a number of free larvae. The larvae resembled in general form those of *O. volvulus*, length of the few measured 280μ to 345μ, breadth about 5μ, anterior end rounded, nerve ring well marked and situated at about 25 per cent. of the length from the anterior extremity, and tail sharply pointed.

In three infected animals, the blood (10 c.c. or more) was examined for larvae, but without success. In this connection it may be recalled that in blood films from one hundred and sixty-six cattle examined at Accra in 1914, filarial embryos were found in five,

that all these were sheathed and were perhaps embryos of *Setaria labiato-papillosa*, a species which has been found in cattle at Accra (Macfie, 1915), but that no larvae resembling those of *O. armillata* were encountered. The skin of these three animals was also examined, because it was thought that, as in the case of *O. volvulus*, larvae might be present in it. No larvae were found, but it must be admitted that considerable difficulty was experienced owing to the thickness, density, and hairiness of the skin, and that consequently the examination was not a very satisfactory one.

J. W. S. Macfie.

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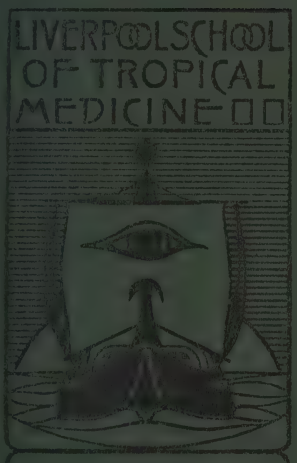

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ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

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A STUDY OF THE TUMBU-FLY, *CORDYLOBIA ANTHROPOPHAGA* GRÜNBERG, IN SIERRA LEONE

BY

B. BLACKLOCK

AND

M. G. THOMPSON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone.*)

(*Received for publication* 15 *August*, 1923)

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I. INTRODUCTION

Although it is more than sixty years since human Myiasis due to the larva of *C. anthropophaga* was first investigated by two French naval surgeons in Senegal, it is a remarkable fact that even to-day complete unanimity as to the mode of infection, the seasonal incidence, the natural reservoirs of the infection, and the appropriate prophylaxis, is by no means attained. It is interesting to find, in respect to this fly and also the South American fly, *Dermatobia cyaniventris*, that a disregard of native accounts has led research into side tracks which have been followed for long periods, before it was discovered that they were leading in the wrong direction. Nothing can better illustrate the manner in which this has occurred than the following extract from the excellent account of Cordylobia Myiasis given by Coquerel and Mondière (1862), who published a paper—the first so far as is known at present—on this subject. Writing of this form of Myiasis as observed by them at Portudal in Senegal, they say 'Cette singulière affection est connue des indigènes, qui savent très bien extraire les larves qui les tourmentent et viennent souvent se loger dans les tissus du scrotum de ces malheureux. Ils prétendent que ces vers sont produits par une petite mouche très commune à Portudal. Cette mouche pondrait ses œufs dans le sable humide, le ver y sejournerait jusqu'au moment où profitant du repos d'un homme étendu sur le sol, il s'introduirait dans la peau de sa victime.'

Unfortunately the authors were so little impressed by the probability of this suggestion that they added 'Il n'est pas besoin d'insister sur les détails de ce récit pour en signaler les erreurs. Il est évident que les larves du Diptère du Sénégal ont été déposés dans la peau, ou que les œufs ont été fixés a quelque poil de cette membrane dès leur origine et que les vers ne peuvent vivre ailleurs.' The theory held by natives in Senegal in those days with regard to the mode of infection by the larvae of Cordylobia, is the same which is held to-day by natives of Sierra Leone, and it is the theory which has been proved to be, in all essentials, correct, by the accumulated research of European observers up to the present day.

In a similar way we find that the work which has been done on the bionomics of *D. cyaniventris* in South America has revealed the

interesting fact that the eggs of this species may be transported on the body of a mosquito and deposited on the animal which is to serve as the host of the larva. It has taken many years to produce the scientific evidence that such a remarkable means of infection can occur, yet the native peasants have known it for so long that their name for the larva is Gusano de Zancudo, *i.e.*, the worm of the mosquito. The suggestion of such a means of infection was treated with frank incredulity by such observers as Da Silva Aranjo, who refers to the peasants' belief in the transference of Dermatobia infection by mosquitoes as 'a popular error, very widely spread throughout Brazil.' This incredulity of those who first investigated the bionomics of Dermatobia had the same effect as that mentioned above, in delaying the discovery of the fact that the mosquito, *Janthinosoma lutzi*, actually does transport the eggs of Dermatobia.

The suggestion has been made by Zepeda (1913) that the larvae of Cordylobia may be carried in the same way by mosquitoes; we have found no evidence of this.

The accurate knowledge displayed by the intelligent African native, the uneducated native who uses his powers of observation to the fullest extent, is often impressive. The Protectorate natives of Sierra Leone quite commonly make for example the nicest distinction between the bionomics of the testaceous flies, *C. anthropophaga* and *A. luteola*. They recognise that the flies are very similar to each other in appearance, and that each produces eggs out of which larvae proceed; they are perfectly aware, however, that of one, the larva lives in the tissues of the host and produces boils, while of the other, the larva merely feeds on the cutaneous blood of the host and lives in the ground, only emerging at night. They can also procure samples of either larva in a short time if required to do so, the former expressed from the larval tumours in the skin of affected animals, the latter obtained from the earth on the floor of their huts by the simple procedure of sleeping on a mat on the floor and searching under the mat in the early hours of the morning. It is, therefore, no more than justice to recognise how in Myiasis, as in very many other diseases, the knowledge of the natives, acquired by the slow and painful process of racial and personal experience, has assisted the investigator to the correct solution of the problems of disease.

II. NOMENCLATURE

From the earliest days of our knowledge of Myiasis due to Cordylobia, that is to say, from the time of the publication of the communication of Coquerel and Mondière in 1862 until the most recent times, when in 1914 Roubaud published his well-known treatise on Myiasis in French West Africa, very various opinions have been held as to the probable relationship which existed between larvae from diverse hosts in different parts of Africa. Coquerel and Mondière, who were not fortunate enough to rear the adult fly, considered the larva to be that of an Oestrid.

Bérenger-Féraud (1872) was successful in rearing flies which were identified by Émile Blanchard as belonging to the genus Ochromyia, Macq.; Blanchard called them by the new specific name, *Ochromyia anthropophaga*. Of this name Austen (1907) says, 'Since, however, no description of the fly whatever was given, *Ochromyia anthropophaga*, Émile Blanchard, is a mere *nomen nudum*, and consequently invalid.' The association of the name *Bengalia depressa* in 1891 with larvae from a human case of Myiasis has proved an additional complication, and still remains so to-day in some publications, in spite of Grünberg's work. Austen, in the paper mentioned above, gives a statement of how the larvae of human Myiasis due to Cordylobia came to be associated erroneously with the adult fly *Bengalia depressa*, Walk. He points out that the type of *B. depressa* is in the British Museum, and that although it is an allied, nevertheless it is a very different, insect from Cordylobia; he says, moreover, that the life history of *B. depressa* is as yet unknown, and that there is not a particle of evidence to prove that its larva is a subcutaneous parasite.

It was Grünberg (1903) who, after a careful examination of all the material available, both larval and adult, came to the conclusion that the fly did not belong to the genus Ochromyia, nor yet to Bengalia nor Auchmeromyia; but was a fly which required for its classification a new genus; he accordingly erected the genus Cordylobia, with the species *C. anthropophaga* (Blanch.). He gave a long and detailed description of genus and species. Dönitz (1905), in an article entitled 'Über eine neue afrikanische Fliege mit parasitisch in der Haut von Ratten lebenden Larven,' gives a description of what he considers to be a distinct species of

Cordylobia, and names it *C. murium*. At the same time, Dönitz reviewed the position with special attention to the ideas expressed by Grünberg and Gedoelst (1905), and shows how the latter came to speak of a *Cordylobia anthropophaga*, Grünberg. Dönitz himself proposed the name *Cordylobia grünbergi* for the East African form.

Roubaud (1914) gives reasons for deciding that *Cordylobia murium* should not be retained as a separate species, namely, that the differences claimed by Dönitz to exist are not sufficiently striking or constant to warrant the species; at the same time the name *Cordylobia grünbergi* is dismissed by him as invalid.

Two other species also are dealt with by Roubaud; of *Cordylobia praegrandis*, Austen, he says that the subsequent discovery of the male has shown that it does not belong to the genus Cordylobia but should be placed in the genus Chaeromyia, Roubaud, while *Cordylobia rodhaini* should likewise be placed in another genus. To sum up, Roubaud says there appears to be only one species of Calliphorines belonging to the genus Cordylobia as defined by Grünberg; that is the fly of Cayor which was first bred by Béranger-Féraud in Senegal, *Cordylobia anthropophaga*, Blanchard. Austen, however, in his summary of the situation, referred to above, ends with the remark, 'The correct designation of this highly important and much misunderstood African Muscid is, therefore, *Cordylobia anthropophaga*, Grünberg. This authoritative statement we have, therefore, accepted.

III. GEOGRAPHICAL DISTRIBUTION

Grünberg (1903) gave as the distribution of *C. anthropophaga* a list of places, which included Senegal, South-West Africa, Gaboon, Dar-es-salam, Zambesi, Lake Nyasa, Tanga, Delagoa Bay, Bagamoyo and Durban. The distribution later given by Roubaud is from Senegal and Lake Chad to the Cape. In the map included in his work, practically the whole region of Africa, south of about 16° North latitude, is shown as infected, with the exception of the North-East area. He points out, however, that the fly is irregularly distributed and that many large areas so far appear to be free of it, or that it has not been recorded from them.

OCCURRENCE OF *C. ANTHROPOPHAGA* IN SIERRA LEONE

Many observations of the clinical effects produced by the larva in man and animals in Sierra Leone have been made in recent years, and this Colony has for many years been considered to be a favourite haunt of Cordylobia. Smith (1908) remarks that 'Tumbu' is a Negro-Creole word, and gives a record of his findings of the larvae in Sierra Leone. He expresses doubt as to the correctness of the accepted mode of infection by the laying of the eggs or larvae in the skin; this doubt arose from his observations of the situation of the lesions in animals. Blenkinsop (1908) noted that in Europeans the upper part of the thigh and the buttock are the favourite site for the larvae to gain an entrance, and it is a generally received opinion that the parasites are often acquired at the latrine. The West Indian troops were often affected in the axilla, and natives, in any region. It was not known in 1908 whether Cordylobia was oviparous or viviparous, but Austen said that in either case, since the female is undoubtedly unable to pierce the skin with her ovipositor, the larva in its earliest stage must bore its own way through the integument by aid of its mouth hooks.

Smith made observations, as we shall see later, on the age incidence of the disease in man and animals, and also succeeded in breeding out flies from larvae obtained from rats and dogs. He mentions a wild rat which had six 'tumbus' in the bare underpart of its legs and feet, which were immensely swollen.

The prevalence of Cordylobia Myiasis in Sierra Leone is so considerable—the parasite itself is the cause of much discomfort to man, and causes suffering and even death in animals—that we took the opportunity of studying as carefully as possible the bionomics of the fly in its various stages, and of making experiments with a view to discovering the best method of attack upon it. We have also made observations upon the morphology of the first instar which may throw some light upon the mode of skin penetration of the larvae of other forms of Myiasis, notably those due to Dermatobia; owing to the fact that we found that the various stages did not always correspond to previous descriptions, we have included a short description of each of the stages of the fly. It is necessary for us to refer frequently to the work of Roubaud, as his

is the most recent and at the same time the most comprehensive work done on the subject. We have been enabled, largely owing to the greater amount of material at our disposal, not only to confirm his observations in many particulars, but also to add to them. If we are compelled to differ from him in a number of points, it is due entirely to the fortunate circumstance that a larger amount of material enabled us to carry our experiments further than he was able to do.

IV. MORPHOLOGY AND BIONOMICS

(1) ADULT (See Plate XV)

The following description of the adult stage is taken from Austen (1908): 'A thick-set, compactly-built fly of an average length of about $9\frac{1}{2}$ mm.; specimens as small as $6\frac{1}{2}$ mm. or as large as $10\frac{1}{2}$ mm. in length are occasionally met with. Head, body and legs, straw yellow; dorsum of thorax and of abdomen with blackish markings; wings with a slight brownish tinge. The eyes meet together for a short distance in the median line above in the case of the male, but are separated by a broad front in the female. On the dorsum of the thorax the dark markings, which are a pair of longitudinal stripes not reaching the hind margin, are covered with a greyish bloom, and, consequently, not very conspicuous; this bloom is also present on the abdomen, but here the markings are much more distinct, especially in the female, in which the third segment, as also the fourth segment with the exception of the hind margin, is entirely black or blackish. In the female, the second segment is marked with a blackish quadrate median blotch, and has a similarly coloured hind border, broadening towards the sides, while the first segment has a narrow dark hind margin. In the male, these markings are not so extensive; the dark hind margin to the second segment is interrupted on each side of the median blotch, which is triangular in shape, and there is a yellow area of considerable size on the proximal half of the third segment, on either side of a blackish median quadrate blotch; the fourth segment is similarly but less conspicuously marked.'

Habits of Wild Flies.

The adult fly material which most observers have had at their disposal has, as a rule, been obtained by the process of breeding flies in the laboratory from larvae taken from the furuncular tumours of animals. Reference in the literature to the capture of adults is extraordinarily meagre. Rodhain and Bequaert (1913) observed wild adult females flying round the cages in which animals were kept, and followed the egg-laying process. Eggs were deposited in the straw and manure in the cages; experimental animals, monkeys and guinea-pigs, placed in the cages where the wild flies had laid, became infected, as the result of the larvae which emerged from the eggs penetrating the skin. In 1911 Roubaud, at Bamako, captured alive one fertilized female which laid eggs in captivity, and which supplied the egg and larval material for his experiments.

We have been exceptionally fortunate in this respect, because we have at Freetown, Sierra Leone, notorious in the history of Tumbu disease, been able to capture many adults indoors. Not only were numerous adult females and males captured, but several of the females were either fertilized before capture, or were fertilized after capture without difficulty. A point of interest is that these captures were effected and the experiments resulting from them were carried out in the dry season, during the months November, 1922, to April, 1923; in its proper place, further reference will be made to the bearing of this fact on the seasonal incidence of Myiasis due to Cordylobia.

On occasions the wild flies were seen on the wing; for example on the 27th March at sunset, on a cool evening several flies were seen in the open darting about after each other and buzzing loudly; they dashed into objects blindly, and one, a female, which had injured itself in this manner was captured.

Natives were able at times to capture adults in their houses, but the construction of their houses taken in conjunction with the resting habits of the fly as observed by us, explains the lack of success which often attended the efforts of the native to capture flies. The flies captured by us were found resting on the dark green painted ceiling of the bungalow verandah; on bright sunny days as many as three or four would be found there; on cloudy days they were rarely

found. They would remain there motionless for long periods, and only when disturbed would they fly about with great rapidity; they emitted during flight a loud buzzing noise, similar to that produced by the blow-fly; the noise ceased when they alighted again. Against the dark surface they presented a very inconspicuous appearance, and would commonly be overlooked. It was easy to understand that if this method of resting were followed on the smoky roofs of houses of native construction, the fly would be even less conspicuous. The flies were easily caught with a collecting net, and they gave the impression of being unable to see well in the day, as they allowed the net to approach close to them without taking flight. Wild flies were seen twice at night attracted by the light of a lamp on the verandah; they flew round noisily, knocking themselves against the lamp, and several had their wings scorched and fell inside the chimney. Whether these flies had come from out of doors to the light or had come to it from some resting place indoors is uncertain. It is probable the latter is the case, as after the systematic capture of all flies resting indoors in the daytime had been undertaken, no further captures were made at the lamp at night.

Wild or laboratory bred flies when placed in glass containers such as cylinders or inverted bell jars, of which the upper end was closed with cloth, rested chiefly on the cloth in the same upside-down attitude as did the wild flies on the ceiling. During the day they were rarely on the wing, but in the early morning from seven to nine, and in the late afternoon from four to six, they became very active, flying about, striking the glass sides of the vessel and buzzing audibly. At night they rested much as in the day, but the appearance of light near them at once aroused them to great activity.

Food of Adults.

Roubaud observed a wild female fly feeding on sugar, on pulped fruit, and on ground soiled by urine. We found that both males and females, whether wild or bred, fed readily on banana and pineapple, the females feeding longer and oftener; both sexes also sucked up the juice from pieces of decomposing rat liver, and less readily fresh blood of a rat from a drop exposed on a slide.

RESISTANCE OF ADULTS IN VARIOUS CONDITIONS.

Direct sunlight. Three wild flies were exposed in large test-tubes to the direct rays of the sun during the hours 11 a.m. to 2 p.m. They survived only from fifteen to thirty minutes. The results of this experiment serve to explain the fact that on bright days during the hot hours the flies come indoors to rest.

Dry heat. Wild flies were exposed to varying degrees of heat; they were placed singly in wide test-tubes which were plugged with wool and provided with a thermometer, the tubes were placed in a water bath which was rapidly brought up to the desired temperature. A male kept for thirty minutes at 44-45° C. was still active at the end of the period. On raising the temperature rapidly great restlessness was observed at 50° C., and at 52° C. the fly dropped suddenly dead to the bottom of the tube. The experiment was repeated with two other wild flies, one male and one female. The temperature was raised from 40° C. to 47° C. in two minutes; after one minute at 47° C. both fell dead suddenly. It appears from these experiments that a temperature of about 50° C. is fatal for the fly.

Cold. Four laboratory bred flies, one male and three females, were enclosed in a large tube containing a slice of banana and placed in an ice chest on 16th March, 1923. After an hour in the ice chest at a temperature of from 10° C. to 6° C. all were motionless. Two were removed to room temperature and rapidly recovered; they were then returned to the ice chest. The flies at each subsequent examination were motionless, sitting sometimes on the glass and sometimes on the banana; they were not observed to feed but changed their position slightly, and recovered their feet when shaken down. On 20th March, 1923, the one male died; the three females died on 23rd, 24th and 28th March, 1923, respectively. The powers of resistance to cold and damp are, therefore, very considerable.

OVIPOSITION. As we have seen, Rodhain and Bequaert observed oviposition on straw and manure in animal cages. Roubaud noted that his fly laid eggs on the glass walls of a vessel and on fruit. We found that of various sites on which gravid females in captivity were given the opportunity of depositing their eggs, the one most commonly selected was dry sand which had previously been

contaminated by the excreta of animals, in this case guinea-pigs. This fact was observed on an occasion when three females were placed in a bell-jar containing a guinea-pig in order to determine whether they would deposit eggs on the animal's skin. These flies had up to this moment been lodged in a container where they had access to cardboard, cotton wool, banana, and glass on which to oviposit; none of these sites was apparently suitable for them, as they did not utilize them. Immediately on their being admitted to the bell-jar containing the guinea-pig, two, and after a short delay, the third also, set about depositing their eggs in the contaminated sand with great eagerness and rapidity; eggs were not laid by them on banana leaves, carrot or orange, which were also present. It is perhaps not without bearing on this point that three other females in which ova apparently mature were present, died in the first container without laying their eggs. On three occasions, when sand was available and utilized, eggs were also laid on sites other than sand, but only in small numbers, viz., six on a piece of black cloth, seven on the white cloth cover of the bell-jar, and eleven on cotton wool. On only one occasion was a considerable number of eggs laid on any other material than sand, when contaminated sand was available; this was a case in which wet sand had been provided for the fly; she landed on it and protruded her ovipositor, but apparently found it too wet for her, as she immediately flew off; she laid one hundred eggs in a plug of pink cotton wool which was used as a stopper to the central aperture of the white cloth cover. It appears probable that the result of this experiment has some significance in regard to the wet seasonal incidence of this form of Myiasis in man and domestic animals. Apart from these occasions eggs were always laid in the sand provided for the guinea-pigs. In numerous experiments conducted during the laying of hundreds of eggs, flies could not be induced to leave the sand on which they were laying. The guinea-pigs did not attract them, nor did they oviposit on clean cloth nor on cloth impregnated with human perspiration, the pieces of cloth being placed in their path as they were laying their eggs. Flies on the other hand would not oviposit on sand contaminated with excreta, if the sand was too moist.

Method of oviposition. Generally for some hours, even a day,

before egg-laying commenced, the female could be seen pushing out and withdrawing the ovipositor, and from time to time small drops of clear fluid appeared at its tip. The procedure when ovipositing in contaminated sand was uniform for all the flies observed. The fly, having alighted on the surface of the sand, and having found a suitable area, digs with the tip of the abdomen a small cavity in the sand, backing slightly and curving the abdomen downwards to enable it to do so; the ovipositor is then extruded and pushed into the sand at the bottom of the small cavity. At this time, when the ovipositor sinks into the sand, the two hind legs bring up on either side a few grains of sand against the ovipositor, which is then withdrawn. The hind legs next move rapidly in a horizontal direction to scrape a little sand over the egg deposited in the small cavity, and to smooth the surface. The fly then advances hurriedly a few steps and commences again to dig in the sand, and repeats the whole process; she does not move in a straight line for long, but turns in her tracks frequently, with the result that a small area may be very thickly sown with eggs. The movements of the fly in the later stages of egg-laying often disturb eggs previously laid by it and uncovers them, bringing them to the surface. On cotton wool the eggs were laid on strands about one quarter-inch from the surface.

Batches and number of eggs laid. From the fact that his fly, which laid over one hundred and fifty eggs, died after ovipositing, Roubaud concluded that Cordylobia cannot survive parturition; also he concluded that probably, as the number of eggs laid was much higher than what he found in the case of Auchmeromyia, only one batch of eggs is laid by Cordylobia. Our observations show that at least two batches of eggs may be laid, and that the female does not die immediately after parturition. For example, a wild fly, No. 22, was observed to lay two batches of eggs in captivity. It was captured on 29th January, 1923, oviposited on 1st February, 1923, and again on 11th February, 1923; it died on 16th February, 1923. A laboratory bred fly, No. 38, emerged from the pupa on 23rd February, 1923, and was fertilized while still unfed on the same day by a wild male; she laid the first batch of eggs on 5th March, 1923, and a second on 8th March, 1923; she died on 10th March, 1923. Another laboratory bred fly which emerged on

10th March, 1923, and was fertilized on 11th March, 1923, laid a first batch of eggs on 17th March, 1923, and a second on 20th March, 1923; she died on 24th March, 1923. The number of eggs laid in the first batch varied from two hundred and eighty-seven to three hundred, in the second batch from ninety-four to one hundred and eighty-four. It appears probable that the batch of eggs laid by Roubaud's fly was the second batch.

Dissection of gravid females. Several females which died without laying eggs were examined. One laboratory bred female which emerged on 20th February, 1923 and copulated on the same date died on 3rd March, 1923, without laying; she contained three hundred and four eggs; another laboratory bred female which emerged on 10th March, 1923, and was not seen to copulate, died on 24th March, 1923, containing five hundred and three eggs in different stages of development; a wild fly, with which a wild male would not copulate, died on 23rd February, 1923; she contained four hundred and four eggs.

Rate of Oviposition. On several occasions when females were engaged in laying in the sand, the total time taken in laying a batch of eggs was noted, and also the rate per minute. The total time taken by one fly in laying two hundred and eighty-seven eggs was thirty-three minutes; by another for one hundred and eighty-four eggs was twenty-six minutes. During the time there were several pauses of varying length, and this reduced the average number of eggs laid per minute. On the whole, however, the rate was very constant for all the flies observed. Thus, taking a total of forty-six individual minutes timed among several flies at the time they were ovipositing, the smallest number of eggs laid in a minute was five, the highest eleven, the average per minute being eight. This relatively slow process is against the idea of egg-laying on animals.

Length of Life of Fly in Captivity.

The longest period during which a wild fly lived in captivity was eighteen days; this was a female, which during that period laid two batches of eggs, surviving the last oviposition for five days. Several laboratory bred females lived fourteen days, but only one lived for fifteen days.

(2) EGG

This is white in colour and measures on an average 0·8 mm. in length; it is banana shaped, being almost straight on one side and curved on the other; it tapers somewhat towards one end. On the surface there are longitudinal grooves, and there is also a fine hexagonal reticulation. In eggs from which the larva has emerged it is seen that there is near the smaller pole a longitudinal slit extending about one-third along the flattened surface; through this slit the larva has emerged.

SITE. The eggs were found just under the surface of the sand in which they were laid; in cotton wool also, not on the surface but about a quarter of an inch deep. The eggs adhered in most cases to particles of sand, or in cotton wool to the strands, and could not be shaken off.

HATCHING. For some hours before hatching the egg shows a darker patch towards the more pointed end; as the time of hatching approaches it is seen that this dark patch is in active movement, and it is recognised as the chitinous buccal armature of the larva tearing at the inner surface of the eggshell. By means of this armature the larva cuts a linear opening on the flat surface of the egg near the small pole; as soon as it is possible to do so, it pushes its cephalic end through the aperture, which it proceeds to enlarge by vigorous movements of the anterior body segments. In cases watched throughout the process, it usually took from four to six minutes from the moment when the aperture was first observed till the time when the larva had cleared itself of the eggshell.

RESISTANCE OF THE EGG TO VARIOUS AGENTS.

Room temperature. On glass the larva emerged in three days as a rule. Roubaud found a shorter period on sand, and noted that eggs on wet sand hatched somewhat later than eggs on dry sand.

Incubator at 37° C. If eggs were placed in watch-glasses either dry or immersed in a small quantity of water they hatched in twenty-four to forty-eight hours, the water drying up.

Sunlight. Exposure to the rays of the sun for one hour, whether on glass or on dry or wet sand, did not prevent them hatching within four days. Two larvae in this experiment were watched

leaving the egg; the process was short, less than a minute elapsed before the larvae were delivered from the egg, but they dragged the eggshell about for another half minute, before getting rid of it. Eggs exposed to the sun for four days did not hatch, even when subsequently removed to the shade at room temperature.

Dry heat. Numerous experiments were carried out with eggs in plugged tubes placed in a water bath. Exposure to temperatures of 60° C., 55° C., 50° C., and 45° C., for two minutes killed all eggs used.

Wet heat. Similar results were obtained by heating eggs submerged in water in tubes placed in a water bath at these temperatures for two minutes.

Hot ironing. Eggs were rendered incapable of hatching by passing over them lightly a flat iron at a temperature suitable for pressing clothes. The eggs were not protected by being in cotton wool, nor even by three folds of cotton cloth; they were flattened and desiccated in the process, a point of some practical importance in view of the frequently accepted theory that clothes are infected when at the laundry.

Cold. Eggs placed in an ice chest did not hatch in seven days, nor after removal to room temperature. Four eggs were placed in the ice chest for forty-eight hours; on removal to room temperature two emerged in three days.

Eggs dissected out of dead females did not develop in any medium, either at room temperature or at 37° C. in the incubator.

(3) LARVA

First Instar. Many descriptions of larvae from cases of Myiasis have been made from larvae which were in the later stages of development. It is, however, very important that not only the later instars should be examined, but that the first instar should receive attention. At this stage, in those larvae which can produce true cutaneous Myiasis, very interesting adaptive structures are found, some of which appear to render the larvae capable of penetrating unbroken skin, while others determine the ability of the larvae either to remain in situ in the skin or to penetrate further into the tissues. The structures which attract attention chiefly are the cephalo-pharyngeal skeleton and the cuticular spines. The first

stage larva of Cordylobia presents points of interest in respect to both these structures, as will be seen in the description given below. Phenol was used as a clearing agent.

The newly hatched larva is white in colour and is visible to the unaided eye. It measures from 0·75 mm. to 1 mm. in length; it is somewhat fusiform, tapering from the mid region towards the anterior, and to a less degree towards the posterior extremity; it is composed of thirteen segments. The first or cephalic segment is the smallest; the mouth aperture is situated near the ventral surface of this segment; the ventral surface of the segment adjacent to the mouth is yellowish, chitinized, and densely clothed with yellow spines directed backwards. On the dorsal region of the segment there are anteriorly two rounded projections, one on either side. On the posterior portion of each projection there is a minute antenna-like structure consisting of two segments: on the anterior portion is a small chitinized pit. Near the caudal margin of the segment are several rows of backwardly directed yellow spines. Segments number two to eight are covered with backwardly directed spines, almost colourless except towards the cephalic margin of each segment where the spines are distinct and more heavily chitinized, yellow or even brown in colour. Segment nine is almost devoid of spines, being provided at the cephalic margin with a single row of backwardly directed spines and at the caudal margin with a single row of spines, in this case *forwardly* directed. Segments ten and eleven have no spines at the cephalic margin, but on the caudal margin have several rows of spines directed *forwards*, the rows being more numerous on the dorsal aspect.

Segment number twelve is longer on the dorsal than on the ventral aspect; it is densely clothed all over with large, strongly chitinized and *forwardly* directed spines. These large spines directed forwards and strongly chitinized appear to act in keeping the larva in position with its posterior end at the surface of the skin. It is interesting to note that, judging from the drawings in Surcouf's (1913) article of the first instar larva of *Dermatobia cyaniventris*, a similar arrangement exists there. This segment in Cordylobia is furnished with several soft digital processes; of these two are visible on the dorsal surface, one on either side of the middle line, two are situated laterally on the segment,

one on each side, while two are situated on the ventral surface, one on each side of the anal orifice.

Segment number thirteen is small and has only a few sparsely distributed spines; on this segment there are four pairs of soft digital

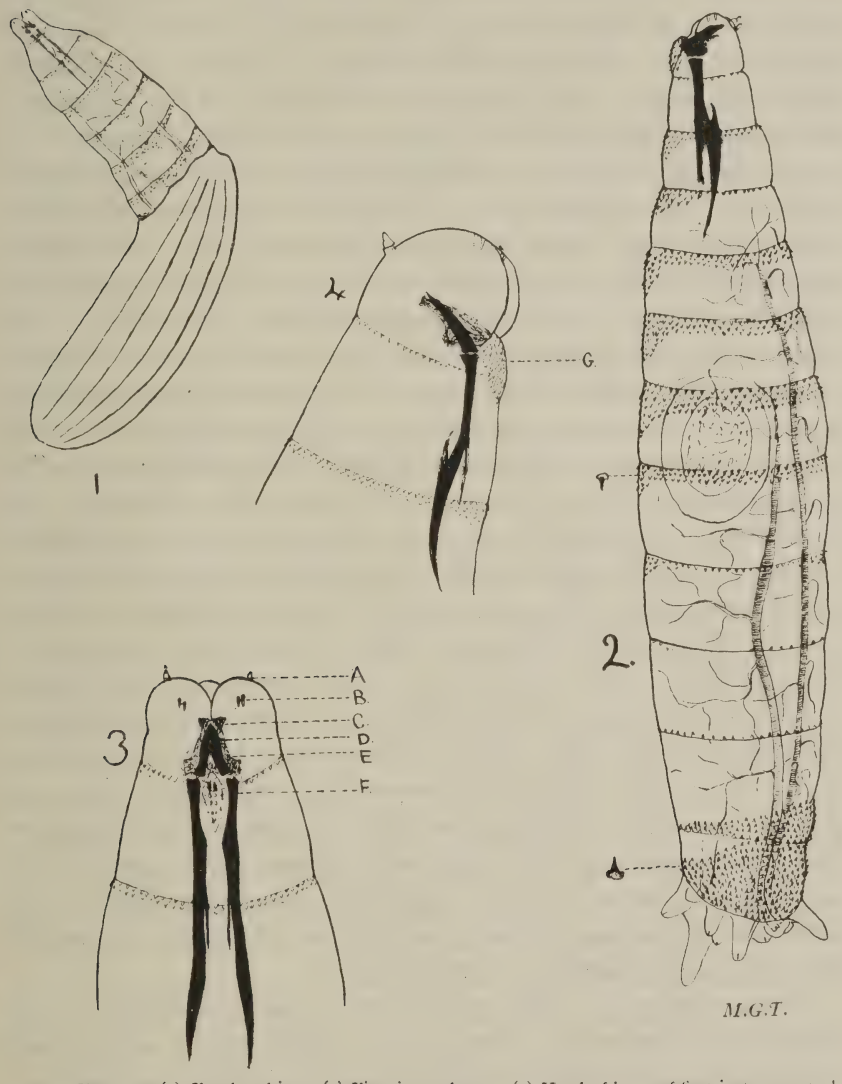


Fig. 1. (1) Egg hatching. (2) First instar larva. (3) Head of larva of first instar, ventral view. (4) Head of first instar larva, lateral view.

A—antenna, later papilla; *B*—chitinous pits; *C*—prestomal sclerite; D—median buccal spine; *E*—oral rods; *F*—cephalo-pharyngeal sclerite; *G*—ventral spiney area adjacent to mouth.

processes in addition to the tracheal tubes; the tracheal tubes open dorsally on the segment near its anterior margin on flattened eminences. The digital processes have in many cases at their tips a chitinous pit; the processes are of assistance to the larva in locomotion, and it is by means of them that the larva can attach its posterior extremity to particles of soil or other objects on which it rests and holds itself erect in the air, while waving its anterior end about in search of a host. The arrangement and position of these processes, fourteen in all, on the last two segments can be seen in figure 4.

The buccal armature or cephalo-pharyngeal skeleton is adapted for penetrating unbroken skin. It consists of a median buccal spine heavily chitinized, which articulates posteriorly with the cephalo-pharyngeal sclerites. On either side of the spine are placed the feebly chitinized oral rods (baquettes orales of Keilin). The cephalo-pharyngeal sclerites are long and slender, and have poorly developed dorsal and ventral cornua. The median buccal spine has received various names, for example, median hook, median tooth, and labral sclerite; according to Keilin, ' the median hook occurs in the primary larva of nearly all Cyclorraphous Diptera.' In Cordylobia first instar larvae, the spine when at rest is directed upwards almost at right angles to the cephalo-pharyngeal sclerite, and when in action its movements are directed upwards to lift the cuticle. Neither in its shape, nor direction, nor in its action, has it any resemblance to the mouth hooks of the second and third instars, which are, according to Lowne (1890), represented in the first instar larva by the oral rods. This median buccal spine is not present in the first stage larva of Auchmeromyia, which larva does not penetrate the skin; it is significant that it is present in *Hypoderma bovis* and *lineatum*, and is figured by Laake (1921), and that it persists in them up to, and including, the third instar. The retention in this case is easily understood when we consider the active migratory character of these stages.

The respiratory system consists of two main tracheal tubes which commence at the posterior stigmata on the thirteenth segment and run forward as parallel bilateral tubes; up to the tenth segment they are yellow in colour; in the region of the eleventh segment there is a transverse tube connecting them, which is also yellow in colour. Along their entire course forward they give off branches, and thus

they become attenuated anteriorly; no anterior stigmatic opening can be made out.

Habits of the first instar larva. The larvae remain in the situation in which they hatch out just below the surface of the sand; they are difficult to see, and even close inspection of the surface with a hand-lens may fail to reveal their presence. If, however, the container in which they are present is vibrated by tapping it, or by shaking the table on which it stands, or if the surface of the sand is disturbed by blowing on it or touching it with a needle, the larvae make their way up rapidly through the few grains of sand covering them. Similarly they come quickly to the surface and wave about, if a vessel containing hot water is brought near the surface of the sand in which they lie concealed. When the larvae are so disturbed it is easy to observe them with the naked eye; they adopt a characteristic attitude, being attached by the posterior end to a grain of sand, the rest of the body being raised in the air, and waving about actively as if seeking for something to which to attach themselves. If any object is allowed to touch the larvae they at once adhere to it and quickly crawl up on it. Camel's-hair brushes were used at first for picking up the larvae, but the larvae have the habit of at once creeping in between the hairs and disappearing from view in a few seconds; brushes were, therefore, discarded in favour of handled needles for the purpose. There is need for great care, however, that they are not injured in transferring them from the needle on to any other object such as a skin surface, as the slightest accidental pressure may render them very slow in penetrating skin or even incapable of doing so.

Resistance of larvae of the first instar to various agents.

Room temperature. Left in sand at room temperature, larvae lived without food for about nine days, as a rule; some died much earlier, and a few lived as many as fifteen days.

On cloth. Larvae two days hatched were taken up on cloth by laying it gently on the surface of sand containing larvae; the cloth with larvae adherent to it was kept at room temperature; the larvae lived on the cloth for nine days; a portion of wet cloth was used to pick up larvae, and allowed to dry with the larvae on it; the original condition of the cloth in this respect made no perceptible difference in the length of time larvae could live on it.

Direct sunlight. Larvae on a watch-glass exposed for twenty hours to open air where the sun reached them during the whole day did not die, and were capable of penetrating skin. Larvae in sand exposed to the sun for two hours in the heat of the day on one occasion only, and thereafter kept at room temperature, lived for over eleven days.

Dry heat. Larvae were placed in small dry tubes plugged with cotton wool; the small tube was placed in a test-tube large enough to contain also a thermometer, and the large tube was placed in a water bath. A range of temperatures and exposures was tried, and it was found that rapid definite effects were obtained at a temperature of 50° C. and above. After two minutes' exposure at 50° C. the larvae became motionless and failed to recover.

Incubator at 37° C. On dry sand in a watch-glass the larvae lived for three days only.

Hot water. In this experiment the small tubes containing the larvae to be tested were filled with water and plugged, and placed in a large test-tube half full of water, which was placed in the water bath. For this series forty-three larvae were used at various temperatures. They survived one minute at temperatures below 48° C., but one minute at 50° C. and higher temperatures killed them. Temperatures from 45° C. to 48° C. for two minutes gave irregular results.

Cold. Larvae placed in a watch-glass and laid on ice, and kept there for twenty hours, became motionless but did not die; on removal to room temperature they quickly became active and were able to penetrate the skin of a guinea-pig.

Immersion in cold tap water. Larvae attached to needles were sunk in tubes of water, and frequently remained attached to the needle for long periods. Consecutive immersion for ten and thirty minutes produced no result; larvae immersed for ten minutes and allowed to dry, and then put in water for thirty minutes, were active at the end of the time; one made an unsuccessful effort to penetrate skin. For longer periods this method was inadequate as the larvae floated up to the surface of the water, where some of them remained active for three days. Complete immersion in tubes for long periods—in one instance up to twenty-four hours—was not fatal to them.

Ironing. The process of ironing was fatal to larvae in cloth, even when covered with several layers.

Phenol. Solutions of phenol of a strength greater than 12 per cent. killed larvae so quickly that they were unable to crawl out of the solution: solutions of less strength did not prevent them crawling out. Watch-glasses were discarded in the subsequent experiments, which were carried out as follows:—A drop of the reagent to be tested was placed on a slide, the larva placed in it, and over the larva a small square of filter paper soaked in the reagent was placed; the filter paper diminished the activity of the larva in crawling out of the fluid. Ten per cent. phenol and 5 per cent. phenol killed larvae in five minutes, while 1 per cent. sometimes failed to kill in ten minutes and frequently failed to do so in five minutes.

Sodium hydroxide. Solutions from 20 per cent. down were tried; all strengths down to and including 1 per cent. killed in five minutes.

Formalin. Five per cent. solution killed in all experiments in ten minutes, but not always in five minutes; 1 per cent. did not kill in ten minutes, but did in twenty minutes.

Chloroform water in the strength of 40 per cent. chloroform killed in ten minutes, but not always in five minutes.

Calomel powder. Larvae placed on calomel powder did not die, but moved about actively in it for an observation period of forty-eight hours.

The effect on the first instar larva of oily substance is mentioned under prophylactic experiments, and the resistance of second and third instar larva is more properly dealt with under Treatment, as these stages are already lodged in the tissues of the host.

The skin penetrating power of first instar larvae. Experiments made with larvae which had hatched from a few hours to as many as fifteen days previously, showed that they were capable of penetrating the healthy skin of various living animals as long as the larvae remained active. Of the fifteen days old larvae tried, only one penetrated, and that not completely; at twelve days old many larvae penetrated easily and completely. Numerous experiments were carried out on the skin of man, European and native, and on chimpanzee, dog, cercopithecus spp., cat, bush cat, guinea-

pig, wild rat and fowl; in all these cases penetration of the unbroken skin was accomplished. Larvae proved unwilling or unable to penetrate the skin of frog, lizard and python. Where penetration of the skin was successful, great variation was noticeable in the time required for the larva to conceal itself under the skin. The animals in which entry to the skin was effected most expeditiously were very young wild rats, brown or black, and in these, as in other animals, the different regions of the body offered differing degrees of resistance to the boring powers of the larvae. For example, six larvae penetrated the shaved skin of the rat abdomen in from twenty-five seconds to one minute; other six placed on the soles of the feet of the rat required from thirty seconds to two minutes. Again on the shaved skin of the thorax or abdomen of the guinea-pig, larvae penetrated in from thirty seconds to a minute and a half; larvae placed on the soles of the feet required from seven minutes to twenty-five minutes. Occasionally larvae succeeded only in partially penetrating the skin; but in no case observed, where the larva succeeded in concealing itself, did the process occupy more than half an hour.

Method of penetration. When an uninjured larva is deposited on the skin of an animal which is suitable, the larva quickly crawls into the nearest groove or wrinkle in the skin, puts down its head and commences to bore in. The median black mouth spine can be seen in active movement, the cephalo-pharyngeal sclerites moving in unison with it; the movement of the spine is directed to piercing the cuticle and enlarging the aperture on both sides; there is a considerable range of lateral movement of the spine, and a corresponding movement of the sclerites to which it is articulated. Once the entrance aperture is large enough to admit the cephalic end of the larva, the body very rapidly insinuates itself under a thin tunnel of cuticle; the rapidity depending on the thinness and softness of the skin. The action of the mouth-parts and the method of using them were studied very carefully in many experiments; particular attention was given to this, as the result of the observation that in the dead fixed larva of the first instar the median buccal spine was usually directed dorsally, and was not curved down ventrally as are the mouth hooks of the second and third instars. The experiments were carried out by snipping off a

very thin layer of skin from recently killed rats and placing it on a slide under the microscope and then placing on it a larva at a point where the action of the mouth-parts could be followed; if the larva entered at a point not desired, the skin could be manipulated with

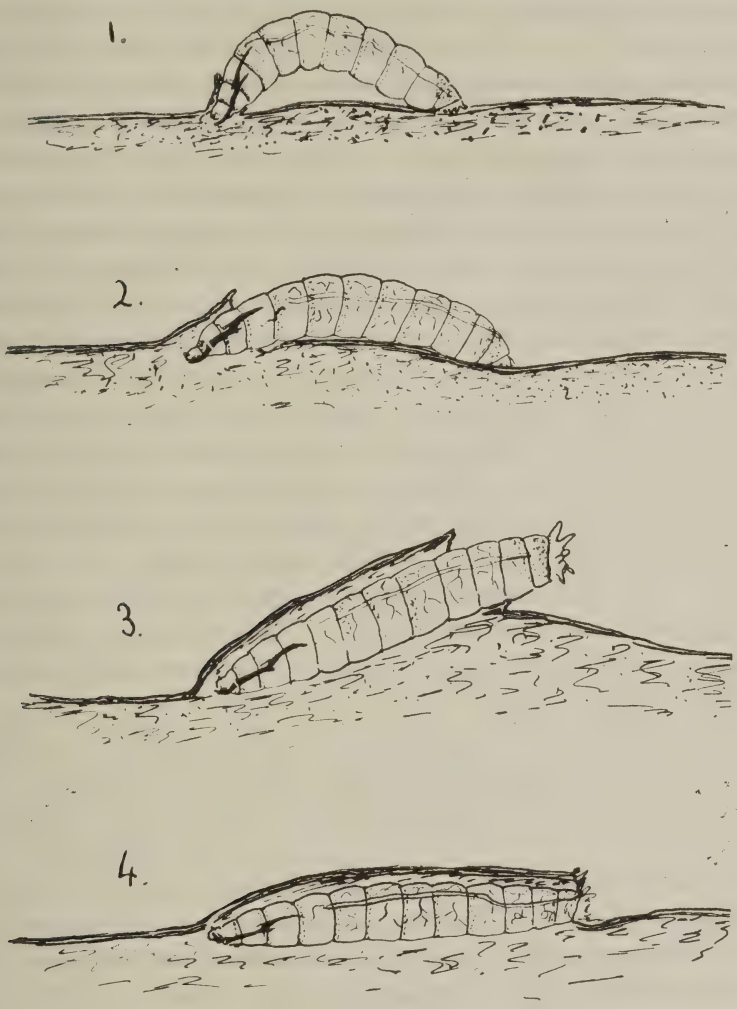


FIG. 2. First instar larva penetrating skin. *M.G.T.*

needles, so as to bring the point of entry into view. In this way it was seen that the median buccal spine was used for penetrating the cuticle and then separating it from the subjacent tissues, by a series of punching movements directed forwards and extending

laterally until an entrance aperture was made; then followed a succession of upward movements of the mouth spine. An illustration which may serve to explain the upward movements is that they resemble the upward movements of the points of a tack-lifter, but in this case, instead of two points, there is only one formed by the buccal spine. In all cases observed, with one exception—a larva penetrated perpendicularly into the ear skin of a dead rat—the first entrance was made into the most superficial layers of the skin in a horizontal direction: the result was that as the larva lay parallel to the surface in a thin-roofed tunnel, the black buccal armature could be discerned through the layer of cuticle covering the larva. It was interesting to see that on the tail of the rat, the larvae, no matter in which direction they faced when laid upon the hairs, quickly turned themselves in such a way that they worked upwards towards the base of the tail. They followed a hair along to its origin from the skin and then proceeded to bore in, sometimes near the base of the hair, but more commonly through the skin between the hairs. The instinctive action of the larva appears to be to obtain as quickly as possible shelter and a resting place in the skin superficially, leaving the further operation of boring in deeply to be done at leisure, when it is securely ensconced and is safe from the risk of being rubbed off by the animal. The posterior segment was invariably left protruding slightly from the aperture, but could be drawn in when touched.

Penetration of the skin of the cadaver. Experiments were made in order to ascertain whether first instar larvae were capable of penetrating the skin of dead animals. In the first experiments the animal used was a young specimen of *Rattus rattus* which had been dead six hours. First instar larvae of various ages were placed on the skin of the ears and tail; in eleven consecutive trials the larvae eagerly attacked the skin and penetrated completely and as rapidly as they did in the living animal. In the second experiments the foot of a dead guinea-pig was used; here again penetration was accomplished with normal rapidity.

Existence in cadaver. Although in no case did larvae which had so penetrated complete their development, they proved capable of living in the tissues of a dead animal for two or three days; on one occasion a larva which had penetrated a cadaver reached the stage

of the first ecdysis. If an animal which had larvae of the second or third instar present on it died, the larvae as a rule at once migrated from the dead body and buried themselves in sand or soil; in the case of one guinea-pig, however, several second and also third stage larvae did not leave the host, but remained active in the tissues for twenty-four hours, before they died.

Secondary penetration of skin. Rodhain and Bequaert removed larvae at an advanced stage from their site in the skin of the host and inserted them into artificially made cutaneous pockets in a different host, and observed that they were capable of developing. Roubaud, as a result of his experiments, concluded that not only older larvae once removed from the host are incapable of penetrating skin afresh, but also that young larvae of the first instar are equally incapable of doing so. This he attributes to a sudden biological modification of the larva. We are in agreement with this observer as regards the lack of *penetrating* power of the second and third instar larvae, but our observations differ from his in regard to the first instar. Second and third instar larvae expressed from cavities in living guinea-pig skin made strong and often successful efforts to regain the position where they had been lodged; they made similar efforts to regain their position in tissues of dead animals on some occasions. They proved, however, quite incapable of penetrating unbroken skin, either of the same animal or of other animals. First instar larvae, however, proved capable of penetrating skin afresh, if removed from the original site within some hours of their entrance; early in the instar they are capable of re-penetration of skin, but later they are not. In Table I are given the details of some experiments.

From the table it is seen that in three experiments with guinea-pigs and rats, first instar larvae succeeded in re-penetrating skin completely; second and third instar larvae failed to do so. We consider that the factor which renders it possible for larvae early in the first instar to re-penetrate, whereas late in the first instar they are incapable of doing so, is the possession in a functional state of the median buccal spine. This apparatus is as well adapted for the purpose of skin penetration, as the mouth hooks of the second and third instars are ill adapted for the purpose.

The effect of reagents in regard to skin penetration. The

cadaver of the young rat was used and powders and oily substances were applied to the skin, and the larvae placed on skin so treated. It was found that French chalk, borax or calomel, delayed them, but did not prevent them penetrating the skin. Oily substances, however, had a great effect, the effect being similar for palm oil, vaseline and liquid paraffin. The larvae placed on skin treated with these did not proceed to bore into the skin; they commenced wandering about, trying to get out of the layer of liquid, and could do nothing as long as they remained in it. The disadvantages, however, are obvious, as the film of oily substance must be of considerable thickness; it is improbable that on such lines a practical prophylaxis can be evolved, as when larvae are free from the liquid they can penetrate the skin, although more slowly.

TABLE I

Giving the results of experiments to test the secondary skin-penetrating powers of the larvae of *C. anthropophaga*, Grünberg.

No. of Exp.	No. of larvae	Instar of larvae	Animal from which removed	Animal on which tested.	Time required for penetration		Remarks
					Min.	Max.	
1	2	3rd	Brown rat	Black rat	...	...	Did not penetrate
2	2	3rd	Brown rat	Guinea-pig	...	...	Did not penetrate
3	2	2nd	Guinea-pig	Guinea-pig	...	...	Did not penetrate
4	1	2nd	Guinea-pig	Black rat	...	...	Did not penetrate
5	6	2nd	Guinea-pig	Black rat	...	...	Did not penetrate
6	1	1st	Guinea-pig	Guinea-pig	...	...	Did not penetrate (end of instar)
7	4	1st	Guinea-pig	Guinea-pig	...	...	Did not penetrate (end of instar)
8	2	1st	Guinea-pig	Guinea-pig	1 min.	3 min.	Penetrated
9	2	1st	Black rat	Black rat	8 ,,	...	Only one completely penetrated
10	2	1st	Black rat	Black rat	2 ,,	3 min.	Penetrated

Food of first instar larvae. First instar larvae grow slightly larger if left in contaminated sand at room temperature, but it is not possible to say whether this increase of size is due to the ingestion of food material which may be taken up in small quantities from the sand. The larvae did not show any capacity for existing

long, or developing on such substances as fruit of various kinds, pieces of muscle, blood or liver of rats. Living tissue affords their natural food.

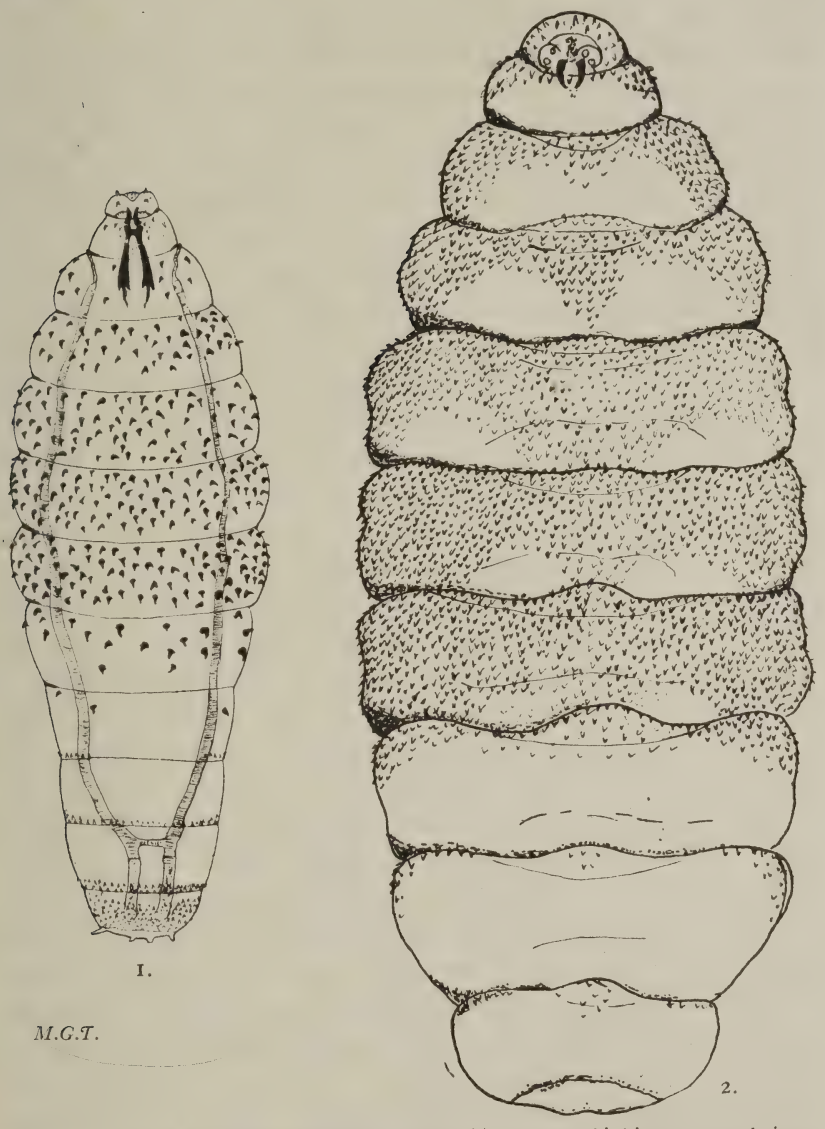


Fig. 3. (1) Larva of second instar, ventral view. (2) Larva of third instar, ventral view.

The Second Instar Larvae. This stage arises by a moult which occurs in the tissues of the host generally about the third day after penetration; the larva then measures 2·5 to 4 mm.; the

appearance and shape of the larva differ entirely from that of the first instar; whereas the first instar larva is somewhat fusiform in shape, the larva of the second instar is club-shaped; it expands quickly from the cephalic end to about the seventh or eighth segment, then narrows quickly and is more or less cylindrical to the posterior extremity. The cuticular spines in this instar are one of the most striking features, being of large size, black in colour, and distributed irregularly over the surface of the third to the seventh segments. The majority of the spines are directed backwards, but spines directed laterally or even forwards occur rarely; some of the spines are bifid. The first two segments bear a few rows of brown spines of smaller size more closely arranged on the ventral than on the dorsal surface. The segments number eight to thirteen, appear almost bare in contrast with the foregoing segments; close inspection, however, reveals the presence of a few rows of small pale spines on the posterior border of each of these segments up to the twelfth, which has numerous rows of similar spines on it. The last segment, number thirteen, which is indistinctly demarcated from number twelve, is devoid of spines; on its dorsal surface the stigmatic orifices open. There are two pairs of processes on the posterior margin of this segment, the outer pair being larger. The anal orifice which opens on the ventral aspect of the twelfth segment is provided with two lateral processes; all these processes are small, compared with the corresponding processes of the first instar. The cephalic segment viewed from the dorsal aspect, presents two rounded eminences, separated from each other by a shallow mesial sulcus. Each eminence has on its upper surface two structures, papilla-like; around the base of each is a brownish ring of chitin, and in the substance of each papilla are what look like delicate chitinous tubes opening on the surface. The papillae are situated one posteriorly and the other anteriorly on the rounded eminence, and each arises in connection with a goblet-shaped structure from the lower end of which a narrow cord passes backwards. On the ventral aspect of the segment the tips of the two black buccal hooks protrude; external to them on either side is a yellow ridge bearing small spines.

The mouth parts consist of two black hooks strongly curved ventrally, in contrast with the median spine of the first instar.

Posteriorly the mouth hooks articulate loosely with a hypostomal sclerite consisting of short rods united by a transverse bar. This sclerite articulates in turn posteriorly with the pharyngeal sclerites which pass backwards, reaching the middle of the third segment in extended specimens.

The respiratory system consists of two main longitudinal tracheal tubes which pass forwards from the posterior stigmata on the thirteenth segment to the posterior lateral border of the second segment, where they end in the anterior stigmata. The posterior stigmata consist of two curved slits, slightly oblique in direction, the concavities of the curves facing each other. The main tracheal tubes are chitinized to about the middle of the eleventh segment; just anterior to the chitinized portion a transverse tube connects the two lateral tubes. In the region of the third segment the main tubes, which have given off branches all the way forward, suddenly narrow and continue as a fine chitinized tube to the anterior stigmata, each of which consists of a fringe of finger-like processes about eight in number. Great variation in size is seen in late second instar and early third instar larvae even from the same host.

Third Instar Larvae. The second ecdysis occurs in the host tissues from the fifth to the sixth days, and the resulting larva matures and leaves the host about the eighth day. The extended larva measured, when mature in rats, from 13 to 15 mm. long. Not only the measurements, but also the date on which the larva reaches maturity and the date on which the ecdysis occurs is influenced by the relative suitability of the host. The mature larva consists of twelve segments; it is roughly cylindrical in shape, its greatest width occurring at the seventh and eighth segments. The first segment is the smallest, and is frequently retracted into the second. It presents dorsally on each side a protuberance carrying two segmented papillae, the basal segment being chitinized. Ventrally on the segment are seen the free extremities of the two black buccal hooks sharply pointed and curved ventrally; on either side of the hooks there is a ridge of yellow chitinized integument bearing a row of small spines, about six in number. The mouth hooks articulate posteriorly with the hypostomal sclerite, which in turn articulates posteriorly with the pharyngeal sclerites; the posterior end of these reaches to the junction between the second and third segments. On

the second segment at its posterior margin laterally, the anterior stigmata open; these consist of a fringe of finger-like processes about ten in number. The twelfth segment is small; near the anterior margin of its dorsal surface the posterior stigmata open close together on rounded eminences; they consist of three sinuous slits obliquely placed; internal to the slits is a small circular opening; there does not exist in this case a definite chitinized plate on which the stigmatic slits open. The cuticle of segments number four to eleven, on the ventral surface, is thrown into folds of an irregular appearance, which are more pronounced in the middle segments, and are concerned in locomotion. Backwardly directed curved spines are present on segments two to nine, being more numerous and of darker colour on the sixth, seventh and eighth segments; on the ninth segment few spines are present, on the tenth still fewer, while the eleventh and twelfth are practically bare. The white appearance of the last four segments forms a marked contrast with the speckled black appearance of the anterior segments. The great diversity of appearance which the larva of Cordylobia presents in its different instars and at different stages of the same instar, has induced some observers to introduce separate names for them; this is of little assistance, and tends to add to the already sufficiently great confusion which exists in regard to the larvae of flies causing Myiasis.

(4) PUPARIUM

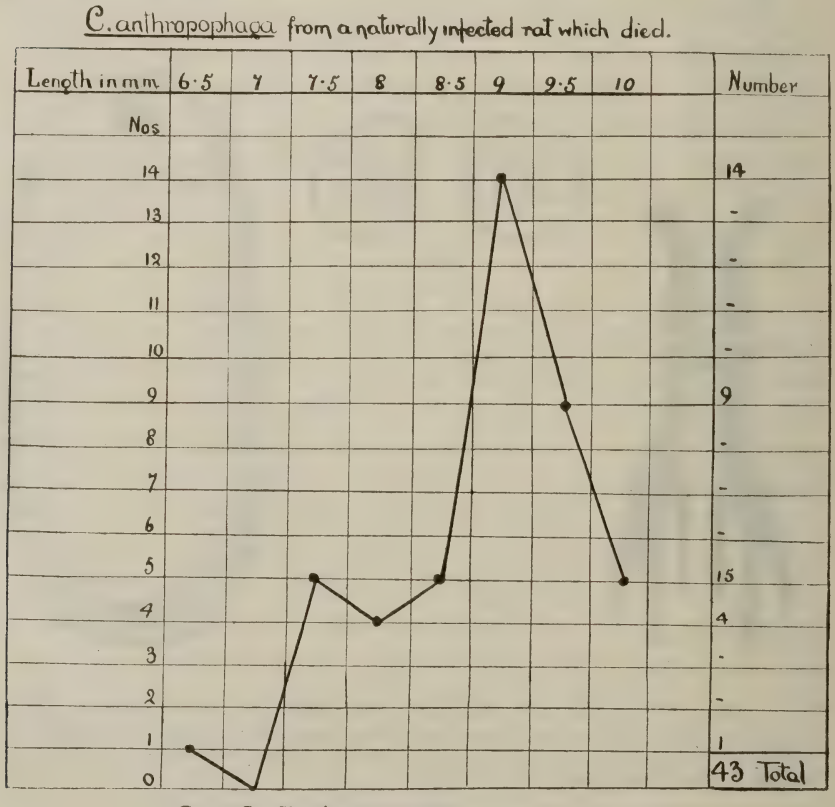
PUPATION. The process of pupation was observed in larvae which had been removed either from naturally infected or from experimentally infected animals. If mature the larvae commence to pupate within twenty-four hours; the anterior extremity becoming pinkish at first, then terra-cotta coloured; the colour extends along the body to the posterior end, and later darkens to a dark chestnut. If the larvae are immature, pupation may be delayed for a day or two, or the larvae may die and turn dark in colour; if the larva has been chloroformed or has been removed from a sloughing septic cavity, it becomes rapidly black from before backwards, the puparium fails to separate and harden, and the larva dies. The shape of the puparium is rather characteristic. It has the posterior end very squarely cut off and the sides run parallel to each other, giving an elongate oblong appearance; it tapers somewhat abruptly

M.G.T.

Fig. 4. (1) Posterior view of first instar larva showing processes. (2) Cephalic end of second instar larva. (3) Anterior stigmata of second instar larva. (4) Posterior stigmata of second instar larva. (5) Cephalo-pharyngeal skeleton of third instar larva. (6) Posterior stigmata of third instar larva. (7) Puparium.

at the anterior end. On the anterior extremity of the puparium are visible two small papillae, and the posterior stigmata can be discerned in most specimens. In all cases the rings corresponding to the segments are seen, and the well defined spines of the larvae are easily made out. When the puparium is treated with phenol in order to clear it, the hard case is still more easily identified as the last larval moult, the mouth hooks and attached sclerites, the posterior tracheal tubes and stigmata, as well as the cuticular spines being readily demonstrable under the microscope.

MEASUREMENTS OF THE PUPARIUM. The smallest obtained measured 6·5 mm.; it was derived from a rat which died before the larvae were mature; the largest obtained was 11·5 mm. long; it was derived from an experimentally infected wild rat in which the larvae matured. Below is shown the distribution according to length of a series of forty-three puparia derived from the naturally infected wild rat mentioned above.



GRAPH I. Showing the distribution according to length of 43 puparia of *Cordylobia anthropophaga*, Grünberg.

Site Chosen for Pupation. Larvae which had been removed from the host, or had left the host, were observed; when placed on sand which was dry they began after a short time to dig down into it, and in most cases in an hour or two they were out of sight. Sand was then provided in small tubes for each larva; the sand was so arranged that there was a damp layer of sand at the bottom of the tube of one inch in depth, and a dry layer of sand above this half an inch in depth; over fifty larvae were tested, and it was found that with a few exceptions all went right to the bottom of the tubes to pupate; the exceptions reached only the top of the damp layer. Occasionally pupation occurred on a dressing applied to an animal's limb over a tumbu lesion.

Experiments with Puparia.

At room temperature. On 16th February, 1923, nineteen puparia derived from the larvae of a wild rat were placed at room temperature; of these seventeen emerged either on 26th or 27th February, 1923. One pupa failed to give rise to an adult, and one adult died while emerging backwards; it may be noted that on several occasions the fly was thus inverted in the puparium. Newstead (1907) drew attention to this occurrence in the case of *Auchmeromyia luteola.*

In the ice chest. On 16th February, 1923, twelve pupae from the same source were placed in an ice chest. None of these had emerged by 9th March, 1923; they were then placed at room temperature, and of the twelve, nine emerged on 14th March, 1923; the other three failed to emerge by 10th April, 1923, when the experiment was terminated. The resistance of the fly in the pupal stage to cold is of interest, as is also the marked prolongation of the pupal stage under these circumstances.

In Incubator at 37° C. On 16th February, 1923, twelve pupae from the same source were placed in the incubator at 37° C.; on 2nd March, 1923, none had emerged; eight were removed from the incubator and placed at room temperature, four being left in the incubator. Of the eight removed, four were kept dry and four were kept moist. On 9th March, 1923, none of the twelve had emerged; the four remaining in the incubator were placed at room temperature, dry. On 10th April, 1923, none of the twelve had emerged and the experiment was terminated. The resistance of the fly in

the pupal stage to dry heat is therefore small. A similar observation has been made by Dove (1918) on the resistance to heat of the pupae of *Gastrophilus haemorrhoidalis*.

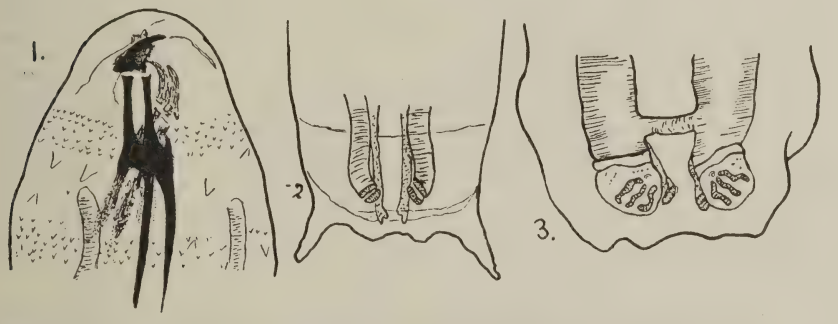
Temperature and dryness. In order to determine, if possible, whether the failure to emerge at 37° C. was due to the effect of temperature or dryness on the pupae, the following experiment was carried out with six pupae derived from a natural infection in a mongoose. Three pupae which had pupated on 19th and 20th March, 1923, were placed at room temperature in a calcium chloride desiccator. Three adults emerged on 1st April, 1923, from the three pupae.

Three pupae which had pupated on 21st March, 1923, were placed in the incubator at 37° C., on 11th April, 1923, none had emerged; they were taken out and placed at room temperature; on 16th April, 1923, none had emerged and the experiment was stopped. So far as these experiments go, it appears that it is the height of the temperature and not the lack of moisture which prevents the development of Cordylobia at 37° C.

Method of Exit of Fly. The puparium is broken at a short distance from the anterior end, and the whole cap or segments of it come off. The fly on emerging pushes vigorously through any resistant substance to escape; it was interesting to see that where puparia had been placed on a plug of wool half-way down a test-tube, plugged also at the mouth with wool, the flies which emerged most frequently went downwards through the wool on which the puparium was lying so that finally they arrived at the bottom of the test-tube. The newly emerged fly possesses great powers of forcing its way through such substances as sand, and cotton wool. As soon as it emerges, it sets about the operation of getting out of its immediate environment. The pressure exerted by the fly was very remarkable; in many cases two inches length of tight cotton-wool plug in the mouth of a test-tube being insufficient to prevent them escaping; in a few instances where the plugs were used, flies had flattened themselves between the plug and the glass to such an extent that they died, with the ptilinum extended in front of them and the wings still unexpanded. Dove (1918) carried out some experiments with *Gastrophilus haemorrhoidalis* puparia, which also showed the capability of the adults to push their way through

obstructing material. Thirty-two puparia were placed in moist loam at a depth of five inches; of these twenty-nine emerged normally, the adults pushing their way up to the surface.

ECDYSIS. The insect from the time it hatches out of the egg as a larva until it emerges from the puparium as an adult fly has undergone four ecdyses. The larva undergoes the first and second ecdyses in the tissues of the host, the first occurring from the second to the fourth days, and the second from the fifth to the sixth day. These dates were determined by removing larvae from their site in the skin of experimental animals at regular intervals after their first entrance, and examining them. The skin is cast from before backwards; the new stage emerging from the anterior end; we have been fortunate in finding larvae at various stages of the moulting process; for example, some specimens showed a condition in which



M.G.T.

FIG. 5. (1) First ecdysis, cephalic end. (2) First ecdysis, caudal end. (3) Second ecdysis, caudal end.

successive stages of the buccal armature were present together, while the posterior stigmata were still only in the earlier stage. Other specimens had the anterior portion of the cast skin loosened and crumpled backwards, while the posterior extremity of the cast was still firmly adherent, and showed the posterior stigmata of the successive stages co-existing (see fig. 5).

The third ecdysis occurs when the fly pupates, the larval skin not being got rid of, but remaining to form the puparium; the pupa which arises inside the puparium undergoes an ecdysis; this constitutes the fourth ecdysis. The pupal sheath cast off is found in the posterior end of the puparium when the fly has emerged.

Pupae removed from the puparium a little time before the time for the fly emerging were found to be enveloped sometimes completely and sometimes partially in the pupal sheath. In some cases it remained only as a delicate covering on the legs.

SIZE OF ADULTS. In the experiments related above, where certain pupae were subjected to the temperature of an ice chest, and in which the pupal period was thereby prolonged greatly, the size of the emerging flies was not appreciably affected. Of the flies which emerged in the experiment, twenty-two were measured: the males, ten in number, measured from 8 to 10·5 mm.; the females, twelve in number, measured from 8·5 to 10·5 mm. It is noteworthy that the size of the larva is no accurate guide to the size of the resulting puparium nor of the emerging adult; it is only a guide within rough limits. For example, two larvae gave the following figures:—

	Size of larva		Size of puparium	Size of adult
	Contracted	Extended		
1	10·0	11·5	9·0	8·5
2	9·5	11·0	10·0	10·5

The size of the emerging fly depended, however, on factors of which the degree of development of the larva is clearly one. Larvae which are removed early and which pupate give rise to flies which are small, while larvae which mature and pupate naturally give rise to large flies. In Plate XVI are shown two series, the first males and the second females, which demonstrate clearly the great variations in size which the fly may present.

FERTILISATION OF THE FEMALE. Copulation took place at once when recently emerged females were placed with wild male flies; it did not matter whether the females had fed or not; copulation lasted about two minutes and was repeated several times during the first day: after that it was not seen to occur; newly emerged males did not copulate readily.

V. DEVELOPMENT OF *C. ANTHROPOPHAGA* Grün. IN ANIMALS

(1) DURATION IN EXPERIMENTS

In the table given below are shown the various stages of the fly as it occurred in two of the experimental animals, a guinea-pig and a wild rat.

It is seen from the table that the development times in these cases were nearly the same, but the regularity with which the larvae in the rat completed the development was greater. As a rule, we found also that adults from rat puparia, as well as the puparia, were on the average somewhat larger than those from guinea-pigs.

(2) PATHOGENICITY TO ANIMALS

In Nature. The animals most commonly found by us to be affected were wild rats, both brown and black; the feet, the genitals, the tail and the axillary region were chiefly involved where single larvae were present; in heavily infected animals any site was apparently suitable, including the nose. Dogs were also affected, and other animals more rarely. Lesions of the feet of sheep and goats were attributed by natives to the larva, but we did not find evidence of infection in those examined—about fifty. Various animals presented old scars and also suppurating sinuses which from the appearance might have contained larvae. The resulting lesions produced by the larvae during growth are illustrated by the case of a wild rat which had torn part of the skin off its abdomen in endeavouring to get rid of the larvae. As a rule, however, animals even with many larvae present either would not, or could not, get rid of them even when in quite accessible places. Several rats and a mongoose died apparently as the result of natural infection.

In Experiments. Guinea-pigs which were allowed to walk on infected sand soon showed signs of infection in the papule formation on the feet; by the third day the feet began to swell and the animals were seen biting them; in two days more great oedema of the feet was present, and in the oedematous skin the larvae could be seen; the posterior end was below the surface as a rule, and the circular aperture was smooth and polished on the margin; the

TABLE II.

Giving the development of *C. anthropophaga* in Guinea-pig and Wild Rat.

Animal	Larva Number	Days in skin	Days to pupation	Days to emergence of adult	Remarks
Guinea-pig ...	1-6	3	...	...	Removed for experiment
	7	8	...	...	Did not pupate.
	8	8	10	22	
	9	8	11	22	
	10	8	11	...	Did not emerge.
	11	8	10	22	
	12	8	10	22	
	13	8	10	22	
	14	8	11	22	
	15	8	11	22	
	16	8	10	...	Did not emerge.
	17	8	12	23	
	18	8	10	22	
	19	8	...	...	Larva lost.
R. rattus	1	8	10	23	
	2	8	10	23	
	3	8	10	23	
	4	8	10	23	
	5	8	10	23	
	6	8	10	23	
	7	8	10	24	
	8	9	11	24	
	9	9	11	24	
	10	9	11	24	
	11	9	11	24	
	12	9	11	...	Pupa preserved.

posterior stigmata are easily seen with a hand-lens at this stage, and also the more striking tracheal tubes leading forward from them, which appear as two parallel silvery lines. The cavities occupied by the larvae are of some depth, because often the posterior end of the larvae is not flush with the skin surface, but lies several millimetres below this; as the mature larva may measure up to about 15 mm., it is seen that the larva may reach with its mouth parts a point about 2 cms. from the skin surface. One guinea-pig in this experiment died as the result of the infection; the larvae were found to have penetrated to the tendons of the feet; the overlying tissues were very greatly thickened owing to the oedema. In one guinea-pig where a larva had attacked the abdominal skin, it had penetrated so deeply as to cause a tumour pushing inwards the parietal peritoneum, which was congested deeply and thickened. Wild rats and small animals frequently died as the result of infection.

In sections cut through the larva in situ in the skin of animals, where no sepsis was present there was noted only oedematous thickening and moderate round-cell infiltration of the tissue surrounding the larval body.

(3) THE ANIMAL HOSTS IN NATURE OF *C. ANTHROPOPHAGA* Grün.

From various sources, beginning at the early observations of Coquerel and Mondière (1862) and coming down to the present day, we have collected a list of animals in which larvae of Cordylobia have been found. In the notes of most observers the two animals which come first are man and dog, the other hosts appearing in the records of a more limited number of observers. Neave (1912-13) mentions that dogs suffer badly, and a case in a rat. It is a very natural thing that especial note should be made of conditions affecting man, and also that any condition affecting the dog should be the subject of remark, owing to the intimate association of this animal with man. It is this natural tendency which explains, we believe, the commonly held opinion that the dog is the usual host in nature of Cordylobia; of this belief we shall say something later. We have found the following animals recorded as hosts:—Man, dog, guinea-pig imported and locally bred, wild rat, various

monkeys, white rat, cat, wild cat, arvicanthis, squirrel, goat and antelope. Roubaud (1914) expresses doubts about the occurrence in goats and antelopes. A case of a mule infection was reported to us, but we had not the opportunity of investigating it. We have found larvae in addition in the mongoose and chimpanzee at Freetown.

(4) THE MAIN NATURAL RESERVOIR OF THE INFECTION

It is a matter of great importance to determine which animal forms in nature the reservoir in which the infection is maintained and from which man derives his infection. Le Dantec and Boyé (1904) wrote, 'Le chien est l'animal de choix pour la culture de la larve.' Roubaud was struck by the fact that dogs are so often affected, as reported by previous workers and also from his own observations. He writes, of dogs, 'Ce sont ces animaux qui constituent le reservoir permanent de la myiase furunculeuse,' and that there is a striking relationship between the human cases and the presence of dogs in the immediate vicinity of those men who are infected. More than this, he concludes that the rarity or absence of dogs in any region is one of the most immediate causes of the absence of the larva from that region. 'Il semble que l'abondance du ver dans un pays soit souvent fonction de celle de la population canine.' The logical deduction which Roubaud makes is in regard to the prophylaxis of Cordylobia Myiasis; as dogs are the natural reservoir, it is against the infection which exists in dogs that man must take action. The action recommended by him consists in the regular inspection of dogs at least once a week, the removal of larvae from them, and the destruction of the larvae. He anticipates considerable results from carrying out this procedure, 'En détruisant ainsi quantité de parasites, à la source même qui les entretient normalement, on arrivera nécessairement à faire disparaître la Cordylobia des endroits qu'elle infeste, ou tout au moins à la rendre extrêmement rare.' There is no doubt but that a careful attention to the expression and destruction of larvae in dogs will diminish the numbers of Cordylobia, and here we are in complete agreement with Roubaud. Where we differ is in regard to the question of dogs being the natural reservoir of the fly; we do not believe that the dog, easily and heavily infected as it doubtless often is, plays such an important part in the maintenance and spread of infection

as to justify the optimism of Roubaud with regard to the results of his suggested method of prophylaxis. We found that in experimental trials, wild rats proved themselves more suitable hosts for the development of larvae than did even young dogs, that in nature wild rats were more frequently and severely infected than dogs, and finally we have been able to prove a close association between Cordylobia and wild rats, by finding in burrows of rats puparia of Cordylobia and rearing from them adults.

In one comparative experiment, using two pups and two medium-sized wild rats, twelve first instar larvae were allowed to penetrate the skin of the abdomen of each animal. Two larvae developed on pup number one, three on pup number two; on rat number one, eleven developed, and on rat number two, eight. The larvae from the dogs left their hosts on the tenth day, those from the rats left their hosts on the eighth day; the average size of the dog larvae was 12 mm. contracted and 14 mm. when extended, of the rat larvae was 12·5 mm. to 14·5 mm.

In nature, apart from large numbers of rats which had a few larvae in their tissues, we observed cases in which very severe infestation caused death. For example in a wild rat, *R. rattus*, nearly full grown, which was brought into the laboratory in a moribund state, there were present in various parts of its body forty-three larvae. The nose, the feet, the genitals, the tail and the general body surface were affected; the animal was in a very septic state and died soon after being brought in. Another rat presented no less than forty-one larvae, and here again the infestation resulted in death.

We have already seen that first instar larvae penetrate the skin of young rats more rapidly than that of any other animal tried; the larvae maintain themselves and develop in a higher percentage in rats than in dogs or guinea-pigs or any other animal used; the time taken by the larvae to develop in the tissues is shorter in rats than in dogs; the larvae which result from the rats are larger than from dogs. So far, then, as experiments are concerned, there is proof that the rat is a more suitable animal host for the larva than is the dog. In nature also we have found the rat more suitable.

We believe that Roubaud himself was aware of this high susceptibility of wild rats from the following three facts. The first

is, that he knew of the observation of Koch in East Africa reported by Dönitz; in East Africa, Koch found a disease in epizootic form which was killing wild rats; this disease on investigation proved not to be due to plague bacilli as first suspected, but to fly larvae present in the tissues; it was, indeed, this fly which Dönitz named *Cordylobia murium*, and which Roubaud is at some pains to prove is, in reality, *C. anthropophaga* affecting wild rats. The second fact is, that in discussing certain observations of Delanoe on Cordylobia in rodents, Roubaud says, 'Elles montrent que ce parasite peut trouver, en l'absence de l'homme ou de gros mammifères domestiques, son réservoir naturel chez de petits rongeurs, avec une électivité très marquée pour certaines espèces à l'exclusion des autres.' The third is, that he made the following experiments:—On page 141 of his work, his experiment B on development in the dog, resulted in twenty-two out of thirty larvae developing on the skin of a dog; he concludes, 'Le chien apparaît donc comme un hôte de choix pour l'évolution du ver du Cayor: c'est la confirmation de ce que l'on constate par l'observation naturelle.' There follows on this immediately, however, his experiment C, on development in the rat; in this twelve larvae developed out of twelve tried on the grey rat (*Mus microdon*), *i.e.*, four larvae on each of three rats. Of this he says, 'La facilité avec laquelle le ver du Cayor evolue chez le rat, fait de cet animal l'hôte qui s'indique le mieux pour l'étude du parasite.' It is remarkable that in spite of the evidence which Roubaud himself produced in the experiments given above, he should have laid such undue emphasis on the part played by dogs in this disease in nature.

(5) TEMPERATURE OF ANIMAL HOST IN RELATION TO INFECTION

Roubaud (1914) observed that in certain species of vertebrates there was a more or less complete immunity towards the larvae. He considers that the development of the larvae is easy in proportion as the temperature of the animal host is low. He gives the following table of rectal temperatures in support of his thesis:—

Rat	36°5
Dog	38°5
Guinea-pig	39°5
Pig	39°5
Fowl	42°0

He found as we have seen that, in experimental work, the rat was a favourable animal, and that the fowl was useless for the development of larvae; we saw that he regards the dog as the natural reservoir of the infection. Man, however, he regards as secondary , 'L'homme ne représente certainement qu'un hôte accidental, chez lequel l'évolution ne se fait pas toujours.' This statement does not accord with the theory that body temperature plays a part of paramount importance in this matter, as the rectal temperature of man, 37·2, is intermediate between that of the rat and that of the dog; from the point of view of rectal temperature man should form a very favourable host. Moreover, our experiments with guinea-pigs showed that they could often be readily and severely infected, and that the infection might even be fatal.

(6) DISCOVERY OF PUPARIA OF CORDYLOBIA IN NATURE

As a result of our laboratory experiments and the observations made on rats infected in nature, we concluded that rats are of great significance in the maintenance of the infection; the larvae found in rats must, we thought, emerge to pupate in the places where rats rested; it was decided, therefore, to investigate rat burrows and search for puparia. Although rats are common in Freetown, it proved no easy task to find rat burrows which were well defined, and which at the same time were situated so as to be capable of being dug out. Dr. W. Allan, W.A.M.S., the Medical Officer of Health for Freetown, kindly rendered us great assistance in this matter, and indicated two accessible rat burrows, one on open ground; the first burrow yielded puparia of Cordylobia; the soil at the entrance was carefully searched before digging was begun; the puparia were found in the first foot of the burrow proper, where the soil was light, dry and friable; from nine of these puparia which were still unopened, adult Cordylobia emerged in the laboratory. The second burrow was in the side of a laterite wall, and one puparium was found here, but the burrow could not be followed into the wall. In addition, puparia were found near the bungalow in rat holes among the rocks, one in each of two localities; from these adults emerged. Emphasis should be placed on the fact that along the river front at Freetown, and also along the small streams through the town, the occurrence of natural hiding places among

loose boulders, clefts in the rocks and unpointed walls, makes it possible for rats to exist easily without the necessity of making definite burrows. This fact will make the thorough survey of rat holes for Cordylobia a difficult operation, and will also naturally render any action against them prohibitive in cost. We are of opinion that these observations not only add one to the already long list of diseases for which the wild rat may be responsible, but also demonstrate that the prophylactic measures against dog infection suggested by Roubaud are unlikely to be as effective in eliminating either the fly or the disease caused by it as he anticipates.

VI. THE AGE INCIDENCE OF THE DISEASE IN NATURALLY INFECTED ANIMALS

It is common knowledge among natives of Sierra Leone that young children are more often affected by Myiasis due to Cordylobia than are grown persons; it is also known by them that young dogs are more frequently and more heavily infested than large dogs. They are so well aware of the fatal consequences which attend infection with the larvae of young pups, that in some places it is regarded as best to drown young pups when heavily infested. Maberley (1918) refers to Dr. K. K. Cross' observation, recorded in Sir Harry Johnston's book on British Central Africa, of maggots in native children—the whole side of a child riddled with holes. Marshall (1902) says that in Salisbury one baby had no less than sixty maggots extracted from it, and that there had been several cases in which babies had had a dozen or more. Grünberg (1903), quotes the observation of Steudel, who in Bagamoyo found larvae regularly on young dogs, but not on grown dogs; in these cases Steudel thinks that the fly seems to like to lay its young on the still soft and moist skin of the new born animal. Le Dantec and Boyé (1904) say ' young dogs above all are severely attacked, and one has sometimes to remove five or six larvae daily for several weeks in succession.' Smith (1908) says ' babies at breast and carried in a cloth on their mother's back are often affected. . . . Small pups suffer more than adult dogs and the larvae are all over them.' Roubaud (1914) mentions that he and Bouet observed numerous cases among dogs in Dahomey, and that at Khombole, even in the

dry season, he found young dogs infested; he adds 'Dans le Baol, j'ai observé de véritables nids de larves chez les petits chiens. Les adultes sont d'ordinaire beaucoup moins infestés.'

NON-DEVELOPMENT OF LARVAE IN SKIN OF LIVING ANIMALS

Although uninjured first instar larvae penetrate with great certainty into the skin of many species of living animal, even when the larvae are many days old, it does not follow that once established in the host tissues they will develop to maturity. This is a matter of considerable significance, and a few detailed observations may be given.

Experiments on Human Skin.

Adult European. 30th January, 1923. A larva 0·9 mm. long was placed on the back of the first phalanx of the little finger at 11.30 a.m.; the larva moved quickly into a wrinkle and it proceeded at once to penetrate, the mouth parts moving very rapidly making an entrance wound; the body pushed quickly into the aperture with a rippling movement, starting from about the ninth segment; no sensation whatever felt. 11.40 a.m.: First sensation felt when body of larva half concealed. 11.45 a.m.: Mouth parts ceased working for a minute; at this time only two posterior segments uncovered; the median mouth spine and sclerites plainly visible through a thin covering of cuticle. 11.50 a.m.: Whole larva concealed in tunnel of cuticle. 1.30 p.m.: Definite constant itching; redness around larva and swelling at situation of anterior end.

31st January, 1923. Definite papule formed; no irritation.

1st February, 1923. Papule larger; no itching, no irritation.

The after history of this larva, as also of one which penetrated the same day on the dorsum of the second finger first phalanx, close to a hair follicle, was that the papule gradually disappeared without further itching and discomfort. Similar experiments were subsequently performed on Europeans.

Adult Africans (Young). In two Africans, experiments were done with four larvae on the inner side of the upper arm.

The history of all these human experiments was similar; the larvae penetrated rapidly and caused irritation and papule formation. After three days they caused no more trouble and the

papules gradually disappeared. Roubaud applied two first instar larvae to his arm, and one to that of Dr. Bouet. The three larvae penetrated normally; as a result there was a partial development only, which went on for twenty-four hours. After this period no development occurred and the small lesions healed easily.

Experiments on Animal Skin.

Guinea-pigs. Eighteen larvae penetrated the skin of the feet of two guinea-pigs; of the eighteen only eight developed; these were removed on the eighth day.

Chimpanzee. Five larvae penetrated the skin of the forearm; none developed; in this case the animal was not prevented from scratching.

Cercopithecus callitrichus. Three larvae penetrated the skin of the tail, but none developed.

From these experiments the information was obtained that larvae which have succeeded in penetrating skin do not always develop; and that this lack of development could not be attributed in most cases to mechanical interference on the part of the animal.

VII. RELATIVE IMMUNITY OF OLDER ANIMALS

Numerous observers, as we have seen, have recognised the fact that young animals are more highly susceptible to Cordylobia infection than old animals. From what we know of the bionomics of the fly and the method by which first instar larvae gain access to the animal body, it is improbable that the comparative rarity of the disease among older animals is due to lack of opportunity of acquiring the infection. It appears clear that the failure of adult animals to show infection is due not so much to non-penetration of the larvae into their skin, as to non-development of larvae after entrance to the skin. The body is capable of resisting the process of development of the larvae but not the entrance of the larvae; the condition is one of relative immunity. If this relative immunity exists, and we have no doubt from observation and experiment that it does exist, we must endeavour to ascertain its nature. It is not an inborn hereditary immunity, because native children and the offspring of animals belonging to the country do not possess this

immunity. It is possible to suppose that it is an immunity which results from age alone, a form of immunity which it is easier to postulate than to prove. Evidence against its being an immunity which arises simply as a result of the maturity of the animal, is provided not only by the first, but by many subsequent observers. Coquerel and Mondière (1862) mention the case of an adult spaniel which was infested by a hundred larvae and died after some days; Bérenger-Féraud (1872) gives an instance of a spaniel which had seventy-eight larvae, while a pup of the same breed had three hundred, and died. Again, adult immigrants to West Africa, involving many nationalities, including Europeans and, as mentioned by Blenkinsop (1908), West Indian troops, are affected by the larvae. It appears improbable that age alone confers immunity. There remains immunity acquired as the result of previous attacks of the larvae, and this we believe to be the true cause of the fact that older animals are less frequently infected than are young ones. Experimental evidence of such an acquired immunity is naturally not easy to produce, as the animals with which experiments are carried out in Tropical Africa are almost certainly partially immune; the repeated entry of larvae to the skin and their subsequent destruction by scratching and rubbing must constantly go on. The following human observations may, however, be quoted. An adult European in Sierra Leone suffered from a natural infection, nine larvae developing in the skin; thereafter he proved resistant to infection at several attempts, as shown below:—

Table III.

Showing the result of attempts to infect a human adult with Cordylobia larvae.

Date	Number of larvae which penetrated skin		Site in body	Result
September, 1922 ...	Natural infection ...	9	Larvae in upper arm	Removed at 6–8 mm. long
January 30, 1923 ...	Experimental infection	2	Fingers	Death of larvae.
February 18, 1923 ...	Experimental infection	4	Arm	Death of larvae.
March 7, 1923	Experimental infection	4	Arm	Death of larvae.
March 30, 1923 ...	Experimental infection	6	Arm	Death of larvae.
April 6, 1923	Experimental infection	12	Arm	Death of larvae.
April 10, 1923	Experimental infection	4	Arm	Death of larvae.
April 23, 1923	Experimental infection	5	Arm	Death of larvae.

Certain animals gave suggestive results. Two dogs which had been infected on the abdomen with partial success, resisted infection at a subsequent date with eighteen larvae each, all of which penetrated. Into the skin of two guinea-pigs which had recovered from an experimental infection there penetrated on 7th March, 1923, four and five larvae respectively; on 15th March, 1923, six larvae; on 20th March, 1923, six larvae; on 23rd March, 1923, six larvae; on 28th March, 1923, four larvae. Although the larvae developed for a period varying from a few hours to two days, in no case did they develop beyond the first instar. A monkey previously naturally infected on the root of the tail received on the perineum on 2nd April, 1923, nine larvae; on 8th April, 1923, six larvae; on 10th April, 1923, six larvae; no development occurred beyond a papule formation which resolved on or before the third day. These instances appear to show that there is an acquired immunity against *C. anthropophaga.*

On the other hand, we encountered certain paradoxical results, as for example, the following:—On 7th March, 1923, a Creole youth received four larvae in the upper arm; development to papules only; on 8th April, 1923, a single larva penetrated and this developed normally. Again, a small dog which had had infection and had thereafter proved resistant to several infections, at the third attempt received ten larvae on 10th April, 1923; of these three developed normally. In each of these two cases anthelminthics had been administered, to the human case betanaphthol before the last infection, and to the dog carbon tetrachloride on the same day as the infection; but whether this fact is merely coincidence it is not at present possible to say.

Against the immunity theory we have also the fact that large rats often present infection; we must conclude from this, either that such rats had not acquired immunity in their early days, or else that their immunity was broken down by some cause late in life. It is not possible, with our present knowledge, to explain why this immunity, as do all forms of immunity, breaks down on some occasions. The broad conclusion, nevertheless, is that there exists among adult animals an immunity against Cordylobia larvae, and we believe that the foregoing experiments point to its being an immunity acquired through previous attacks of the larvae, whether

these developed or were destroyed before developing by mechanical interference on the part of the host.

DURATION OF IMMUNITY

So far as we are aware, there are few facts available as yet upon this point; the following observation of Heckenroth and Blanchard (1913) has some significance and deserves mention. A European in the Congo had a fox terrier which acquired infection in October, 1911; the owner got infected in November, 1911. The owner and the dog left Africa and returned in over a year. In January, 1913, the dog became infected, and in February, 1913, the owner became infected; the immunity if established by the first attack, did not persist for much over a year at the most.

CUTANEOUS REACTION

In the human case, where repeated attempts were made at infection, there was a severe local reaction at each of the last six attempts; the penetration of the larvae was accompanied by great itching and was followed within a few minutes by remarkable local signs. At the point of entrance of each larva a white bleb formed and spread rapidly in all directions; the larvae were placed on an area of about two inches by three, and yet in all cases within fifty minutes the white urticarial wheals had coalesced and produced an irregular swelling about three inches in diameter and raised in the centre about a quarter of an inch; around the white area was a zone of deep congestion which faded away at the margins. The rather tense white swelling stood out in a striking manner against the red background; very considerable itching was felt over the whole area affected, while at the same time a slight pricking sensation caused by the larvae boring was experienced. By next day the swelling, redness, and to a great extent the itching, had subsided.

Hadwen and Bruce (1917) made observations on Anaphylaxis, in cattle and sheep, produced by the larvae of *Hypoderma bovis*, *H. lineatum*, and *Oestrus ovis*. Intravenous injection of larval extracts in saline solution produced death rapidly in some animals, severe reactions in others, while in some it produced no ill-effect. The preliminary sensitisation was not experimental but natural, and

was attributed to the excretions of the larvae; in one case there was evidence of the sensitiveness being inherited. Anaphylaxis was only found to occur when larvae were broken in an animal, or when a dose had been injected. An ocular reaction occurred in sensitive animals when a drop of juice from a larva was placed on the conjunctiva. The cutaneous reaction in our case, while apparently anaphylactic in nature, resulted not as in Hadwen and Bruce's experiments from the injection of extracts, but from natural penetration of larvae into the skin, the larvae being uninjured.

These observations which we have made on immunity appear to us of great importance, not only in so far as concerns Cordylobia Myiasis, but still more in relation to the Myiasis caused by Dermatobia and Hypoderma, in which cases enormous loss is suffered year after year on account of damage to hides. The development of some method of immunizing cattle against the attacks of the larvae would result in a great increase of value of hides from countries which are affected by such larvae. The remarkable thing is that in South America, in the midst of a country in which cattle are severely affected by the larvae of Dermatobia, there actually exists a breed of cattle—Antioquia—which enjoys a relative immunity. We are indebted to Mr. M. T. Dawe, Commissioner of Lands and Forests in Sierra Leone, for showing us his photographs of this breed and for much interesting information concerning it. We feel convinced that a further study of this subject, with a view to discovering some means of artificially producing a definite immunity would well repay the trouble and expense involved.

VIII. SEASONAL INCIDENCE OF *C. ANTHROPOPHAGA* Grün.

The consensus of opinion of previous observers appears to be that the wet season is the season of prevalence of infection with Cordylobia larvae. Coquerel and Mondière (1862) stated that in the month of July, in Senegal, after the commencement of the rains, many cases of these larval parasites occurred. Le Dantec and Boyé (1904) say that in French Guinea, the adult appears in the beginning of the wet season, disappears abruptly in October, only to reappear next year with the first rains. Rodhain and Bequaert

(1913) mention a series of animals which are affected with larvae at Katanga, all in the wet season. Roubaud (1914) refers to Bérenger-Féraud's observation that in human beings this form of Myiasis occurs in July. Howard (1912-13) says that the larva is very abundant during certain seasons in the Transvaal. The infection of human beings is considered by Roubaud to result from close association of dogs with men, the fly being primarily attracted by the dogs. Our experience as regards adult flies is not in agreement with that of Le Dantec and Boyé; during the dry season, as we have shown, the adult fly is not rare in Freetown; we captured over a hundred wild specimens, both male and female; fertilization and oviposition occurred constantly during the dry season. There does not seem to be any reason why infection of man should not occur in this season if the theory that dogs are the attraction which brings the fly near man, and form the main reservoir, were correct. That these flies were not, in this case, attracted by dogs is evident, because there was no dog present in the bungalow; it was equally clear that they were not attracted by the latrines, as in no case was a fly ever captured or seen there; nor were they attracted by the clothes accumulating for the laundry, which they were never seen to approach. They appeared not to have come in to lay their eggs, because males as well as females came in, in about equal numbers and because no female which was captured was actually ready to lay eggs when it came indoors. The correct explanation probably is, in accordance with the experimental evidence on the effects of sunlight, that the flies were simply taking shelter from the heat of the sun; we have pointed out that on dull days they did not come in. The source from which these flies came was, we think, the rat holes in the rocks adjacent to the bungalow, where, as we have pointed out, puparia were found. Rats captured near the bungalow were frequently infected, and sometimes heavily.

It might be argued that, although in this case the flies were not attracted by the presence of dogs, if dogs had been present they would have induced the flies to oviposit in the house; that might be so, but if it were the case, how can we explain the absence of human Myiasis at this time of the year in houses where dogs are kept at all seasons of the year. The fact that there is a definite

wet seasonal incidence of Myiasis in man and also dogs points to some factor at work which is independent of the presence or absence of dogs.

One explanation which suggests itself arises out of our discovery that the wild rat is the chief natural reservoir of the fly; this explanation is, that the seasonal incidence in man and also in dogs is dependent on the seasonal habits of the rat. It is commonly known that in the wet season rats congregate more closely in the neighbourhood of human habitations; this movement is due possibly to the flooding out of their burrows; this theory involves that Cordylobia moves with the rat, and so is brought into close association with human habitations. Another explanation is that it is simply the desire to escape from wet which makes the fly lay its eggs indoors in the wet season.

The flies came in freely, in our experience, during the dry season, but not with the idea of ovipositing; their normal place for ovipositing in the dry season is not indoors. We have seen, however, that in experiments the fly avoids wet sand and will not lay her eggs there, she will rather even lay them on cotton wool and cloth, and this was clearly done in the case of natural infection mentioned below. This fact alone might account sufficiently for the increase in human Myiasis in the rains, and also to a less extent for the increase in Myiasis of domestic animals. It is probable, however, that both factors are at work.

IX. MODE OF INFECTION OF MAN

The earlier erroneous ideas that the fly lays larvae or eggs in the skin of man, or that it attaches its eggs to hairs, have already been referred to. The method of infection in man is by the penetration of the first instar larvae into the skin; for this the larvae must effect contact with the skin. It is clear that there are very many ways in which such necessary contact may be brought about. It is possible, for example, that where soil or sand, especially if contaminated, is used in latrines, the female fly may deposit her eggs in the sand box; if in the act of using the sand some of it is spilt on the seat of the latrine and first instar larvae

are present in the sand, the next person to use the latrine will almost certainly have the larvae penetrate the skin. Again, if flies are hard pressed, they will lay their eggs on clean clothes, and when the larvae hatch out, if the clothes are put on, infection will occur. An interesting case is that reported to us by Dr. Wright, of Freetown, a most accurate observer, who has great experience of this disease; he was called to see a patient who had injured his shoulder; he wished to put it up at once, and for a bandage was provided with a window curtain which had been washed and ironed and had been lying in the house for some time. In a few days, only under the curtain, there developed on the patient's thorax, back and front, and on the arm, thirty-eight larvae of Cordylobia; the other curtain which had been lying underneath the first was examined carefully by us, but no eggs or larvae were present; there appears here the strongest evidence of the infection from clean cloth. The probability of the larvae derived from eggs laid on dirty clothing surviving the washing, exposure to the sun to dry, and subsequent ironing appears very small. Again, in view of the adult fly's dread of the bright sun and the lethal effect which this produces on the fly, it appears improbable that clothes hung up on lines to dry in the sun would have eggs deposited on them; if the clothes were laid on the ground to dry they might pick up larvae easily from the soil, but would not be so likely to pick up eggs which are lying slightly below the surface. In the houses of natives, the occupants could obtain infection by lying on infected soil, and also in any of the previously mentioned ways.

X. SYMPTOMATOLOGY

IN ANIMALS

The presence of one or two larvae produces little obvious distress even in small animals; when the larvae are numerous, however, there is very considerable irritation, and the obvious illness of the animal results chiefly from septic absorption combined with loss of sleep; the appetite diminishes, and the animal loses weight. Where larvae are single there is little to note beyond the localized small tumour; where larvae are close together great swelling and oedema occur, and the tissues intervening between larvae become

sloughing and gangrenous; the removal of individual larvae, then, is difficult, as the whole area of skin surrounding it may come off with them. The larvae are not always confined to the true skin, but often penetrate into the deeper tissues; in the case of some of the guinea-pigs, as we have stated, they exposed the tendons of the feet. In the abdominal wall the parietal peritoneum may be involved, giving rise to rigidity of the abdominal muscles, retraction and tenderness. In certain regions the presence of larvae produces more serious lesions than in others; the feet and scrotum easily become gangrenous; the case of one pup which became blind of an eye was reported to us; the larva had penetrated the skin before the eyes were open.

IN MAN

The actual penetration of the first instar larva into the skin is hardly noticeable; in some cases, like the one referred to in a previous section, an intense cutaneous reaction occurs. Roubaud observed a similar reaction in his own case, and accounted for it by saying that the larva had become contaminated from the soil in which it was. It appears to us more probable that this is a body reaction in response to the presence of a specific substance produced by the larva, possibly of a salivary nature, and that it has some connection with the marked condition of immunity which existed in one of our human cases. Such a reaction was not observed by us in the case of another European, recently arrived in this country; in this case larvae of the same batch introduced themselves into the skin without reaction and proceeded to develop. The larva developing in man is felt during the first two days or so, causing slight itching or pricking at intervals; the symptoms and signs are easily overlooked. The papule which forms, increases in size and becomes red; there is then a more or less complete cessation of symptoms for several days possibly, although the furuncular swelling increases. Then the symptoms recur with greater severity; the pain increases and becomes so sharp as to interfere with sleep. The larva becomes very active at intervals and can be seen clearly retracting into the cavity and then pushing against the margins of the aperture in the skin to increase its size. Much serous fluid may exude at this time; the skin and subcutaneous tissues have meantime

become much indurated and the area round the aperture is deeply coloured; tenderness on pressure exists; the lesion resembles a boil, for which it is frequently mistaken. Gland enlargement may occur and general symptoms, malaise and febrile reaction. The development was slow in human beings observed; in one case a larva removed on the fifteenth day in the third instar measured only 9 mm. The stage at which larvae are usually brought under notice by human beings is after the third stage has been reached, at which time the larva, in enlarging the entrance aperture preparatory to making its exit from the skin, exercises considerable force. The cavity formation is out of proportion to the size of the larva, and it appears as if the larva produced a lytic action on the tissues near its head end; the clear fluid which comes from the cavity at intervals is sometimes stained with blood and also with faeces of the larva. On removal of the larva the symptoms disappear, and healing usually occurs readily.

Nagel (1897) observed larvae in his skin in East Africa for a period of four weeks; but the record is not complete.

XI. TREATMENT

Various methods of treatment were tried experimentally, such as the effect of tobacco smoke, insufflation of calomel powder, French chalk, dropping on tobacco juice, chloroform water, phenol solution and cresol solution in 5 per cent. strength, application of vaseline, palm oil and liquid paraffin. Simple expression was effective in removing the larvae, but often painful; removal by fine forceps was easy in the later stages if the aperture was large. Of these various methods the one finally adopted, especially for use with small larvae which cannot be removed easily with forceps, was the application of liquid paraffin and subsequent expression. Blenkinsop (1908) records the effect of a plaster of sugar and soap in causing larvae to emerge, owing to the blocking of the posterior spiracles. The natives of Sierra Leone use palm oil, the pericarp oil of *Elaeis guineensis*, with the same object; they say, however, that the Tumbu comes out at night to feed on the oil. Palm oil was tried, but was discarded owing to the colour; in its stead liquid

paraffin was used, and acted admirably even with the very small larvae. A film of paraffin is placed over the opening in the skin, any scab being first gently removed; at once the posterior end of the larva begins to come out; the film is then thickened by adding paraffin drop by drop; the larva in its efforts to reach the surface of the film makes greater movements out and in; in doing so it lubricates itself and the walls of the cavity; the superfluous paraffin is wiped off, and the two thumbs are placed a little distance on each side of the aperture and pressure inwards and downwards applied; the larva comes out slowly at first, later with more rapid movement. The larva should be destroyed. After extraction of the larva and healing of the wound, a mark remains for a long time; Fülleborn (1908) could still after ten years see the marks left on the skin.

XII. PROPHYLAXIS

Adults should be looked for daily in houses on the ceiling of rooms and verandah, during the sunny hours of the day; any fly present should be captured in a collecting net and destroyed. All latrines should be fly-proof.

Sand and soil used for the latrine may be heated in a kerosene tin for some time before being placed in the latrine box, in this way eggs and larvae are destroyed.

In affected districts the weekly examination of domestic animals should be carefully done, and any larvae found, expressed and killed; it is most important to destroy the larvae.

Rats should be eradicated as far as is possible from houses and compounds. Those captured should be destroyed by burning before the larvae leave them.

In cases of small boils where there is doubt as to the presence of a larva, the application of a drop of liquid paraffin will cause the hind end of the larva to move actively; in any case liquid paraffin will be of great assistance in removing larvae with as little pain as possible.

Clothes lying exposed are a source of danger; it is especially underclothes and bed linen which are apt to carry infection to man; a very certain method of prevention here is to have all the clothes

ironed after washing and drying, and to store them immediately in covered receptacles.

It is advisable to have such clothes washed in the compound, and after ironing kept in drawers or suitable covered boxes; this simple precaution will prevent flies laying on them.

XIII. COMPARISON BETWEEN *CORDYLOBIA ANTHROPOPHAGA* AND SOME OTHER MYIASIS-PRODUCING FLIES

Myiasis is a wide term embracing parasitism of very varying degrees, and involving widely different parts of the body. We shall confine our attention here to those forms of Myiasis caused by flies which in the first instar have been proved, or appear to be capable of penetrating the unbroken skin.

(1) *BOOPONUS INTONSUS*, Aldrich

Woodworth and Ashcraft (1923) describe a condition of Myiasis affecting the feet of carabaos and bullocks in the Philippine Islands; larvae were reared and adults bred and forwarded to Aldrich. Aldrich (1923) from three females sent to him created the new genus Booponus with the species *B. intonsus*. He refers to the close similarity of the adult to Cordylobia. The description of Woodworth and Ashcraft deals with two larval stages, but the illustration of the young larva shows that it is not like the first instar larva of Cordylobia; rather it resembles closely the second instar of this fly, not only in the appearance of the posterior spiracles but also in the very large and dark spines irregularly distributed over the cuticle.

It is surmised, but not proved, that the first instar larva which arises from eggs attached to hairs is capable of producing Myiasis by penetrating unbroken skin; if it appears later that this is so, we should expect, on analogy, that a first stage larva having a buccal spine will be found.

(2) *WOHLFAHRTIA VIGIL*, Walker

Walker (1920) described two cases of cutaneous Myiasis in infants due to larvae of this fly; evidence was given of a clinical character that these larvae appeared to have entered the unbroken

skin. Again Walker (1922) gives details of another case and also descriptions of the first instar; he says 'the median or labral hook arises from a slightly divided base, immediately in front of the pharyngeal sclerites, and is strongly decurved, the pointed apex projecting slightly from the front part of the oral aperture in the usual position.' The illustration shows well this curved hook; this character differs from the median spine in Cordylobia first instar larvae; the appearance of this apparatus in *Wohlfahrtia vigil* does not suggest that the mode of entering unbroken skin can be the same as in Cordylobia; it is possible that when skin penetration experiments are carried out, it may be found that this larva, if it can in fact penetrate healthy skin, does so by digging at once deeply into the tissue, and not, as in Cordylobia, by raising over its dorsal surface a thin layer of cuticle.

(3) *HYPODERMA* spp.

Laake (1921) gives an account of the anatomy of the mouth apparatus of *H. bovis* and *H. lineatum*; he refers to the fact that Riley (1892) first described the real first stage larva of *H. lineatum* which he obtained from the egg before hatching; he mentions that Gläser (1914) and Carpenter, Hewitt, and Reddin (1914) first observed the first stage larva of *H. bovis* outside of the egg. Laake in his description and drawings shows that the median mouth spine in these larvae is retained not only during the first instar but also actually during the second and third instars, during which the larva is passing through the tissues, and is not cast off until the larva reaches the back of the host. It is interesting to note that the ventral curvature of the spine is relatively slight compared with that depicted by Walker for *W. vigil*, and resembles more the condition present in Cordylobia.

(4) *DERMATOBIA CYANIVENTRIS*

Surcouf (1913) figures the first instar larva of this fly; the larva which is able to penetrate the unbroken skin is possessed of a buccal armature closely resembling that of Cordylobia; the large forwardly directed cuticular spines on the posterior segments which we drew attention to in Cordylobia, exist in this larva also.

XIV. SUMMARY

1. The morphology and bionomics of *Cordylobia anthropophaga*, Grün., have been studied in some detail during the dry season 1922-23, in Freetown, Sierra Leone.

2. Certain new facts as regards the habits of the adult, its method of oviposition and the number of eggs laid by it are recorded.

3. In the first larval stage also, attention is drawn to certain morphological peculiarities, both in the buccal armature and in the cutaneous spinulation, which appear to have a direct and intimate connection with the process of skin penetration.

4. A direct association between Cordylobia and wild rats, which was suggested by field observation and laboratory experiment, has been proved to exist by the discovery of puparia of this fly in the burrow of wild rats.

5. Evidence is produced which appears to incriminate the wild rat as the main reservoir of the infection in nature, and to associate these rodents with such seasonal incidence of the disease as exists.

6. Numerous experiments were carried out both in man and animals, which add considerably to the knowledge of the mechanism of infection, pathogenicity and prophylaxis.

7. An immunity has been proved experimentally to develop against attacks of the larvae, not only in man but in animals.

8. The development of such an immunity in the case of cattle in similar forms of Myiasis is considered a possibility and worthy of investigation.

9. A comparison is made between Cordylobia and some other flies which cause cutaneous Myiasis.

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EXPLANATION OF PLATE XV

Cordylobia anthropophaga. Adult ♂ and ♀.

del. M.G.T.

EXPLANATION OF PLATE XVI

Cordylobia anthropophaga. Photograph of series of adults and puparia.

Photo. by M. Brown

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EXPLANATION OF PLATE XVII

Photograph of naturally infected rats (shaved with Barium depilatory powder).

Photo. by M. Brown

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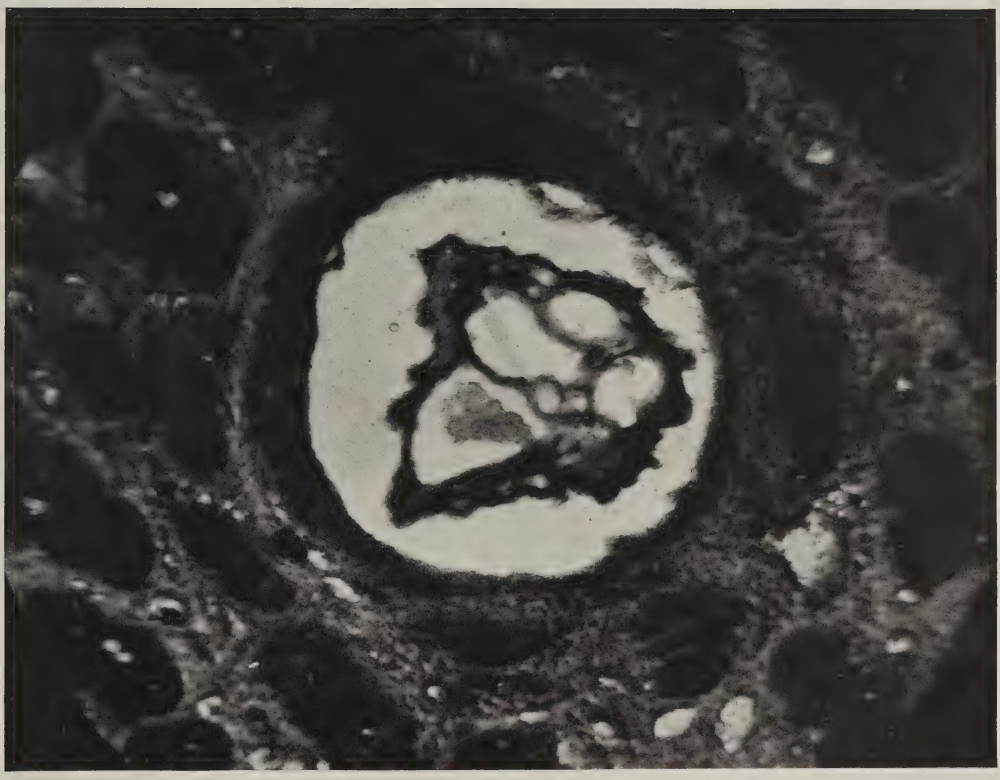
EXPLANATION OF PLATE XVIII

Cordylobia anthropophaga. Photograph of second instar larva in the tissues of a guinea-pig. × 8.



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THE TRANSMISSION OF *T. CONGOLENSE* BY *GLOSSINA PALPALIS*

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I. INTRODUCTION

Representatives of the group of trypanosomes to which *T. congolense* and *T. nanum* belong are widespread throughout Africa. They are common alike in game and in stock. To the game they are, apparently, harmless, but as parasites of domestic animals they constitute a factor of considerable economic importance.

The trypanosomes of this group are well adapted to rapid propagation in nature. For indirect or cyclical transmission they depend, so far as is known, entirely on the *Glossinae*; and, though less common than *T. vivax* and *T. uniforme* in wild game-tsetses, they are considerably more prevalent in the fly than the polymorphic trypanosomes of the *T. brucei* group. In all species of *Glossinae* in which cyclical development of the *T. congolense* group takes place, the flagellates multiply first in the gut of the fly, and later on take up their 'anterior station' in the labrum and hypopharynx of the proboscis.

In addition to cyclical transmission, they are readily conveyed

from mammal to mammal by the direct method—that is, without any alternation of generations. It is probable that most, if not all, the mammalian trypanosomes can, on occasion, avail themselves of direct transmission, but it is doubtful whether any other group of the *Glossina*-carried organisms relies to the same extent on this method in nature.

The so-called species contained within this group have been arbitrarily separated on account of differences in their behaviour in certain mammals. The *nanum*-form cannot infect dogs and monkeys and the small laboratory animals, while the *congolense*-form can; otherwise they are indistinguishable. Both these two forms of the trypanosome have been recovered from game and from wild fly, so that this idiosyncrasy may, to a slight extent, influence the distribution of the parasites in certain regions. But to allow to physiological variations of this kind the importance attaching to a specific character, is, as Yorke and Blacklock (1914) have pointed out, unjustifiable; and, as a matter of fact, strains whose virulence is intermediate between these two extremes do occur in nature. The group should, therefore, be regarded as a collection of strains whose morphology and behaviour in the insect intermediary are uniform and constant, but which vary in their relation to certain mammalian hosts. It has been shown elsewhere by laboratory experiments, that the effect of long-continued direct transmission on a strain of *T. brucei* may be to enhance its virulence in mammals, and, at the same time, to eliminate the power of developing cyclically in *Glossinae*. The same principles probably apply to the group we are considering. There is, indeed, field-evidence to show that some of the more virulent strains of *T. congolense* rely largely, if not solely, on direct transmission, for their passage from mammal to mammal. Whether such strains retain the power of cyclical development in *Glossinae* has, however, not yet been determined.

The gregarious habits of domestic stock afford excellent opportunities for the spread of their trypanosomes by direct transmission, by *Stomoxys* and the other species of biting flies that, in Central Africa, swarm around the unfortunate animals. Among the game this method functions probably much less commonly, although with certain species such as elephant, buffalo and eland the requisite conditions will doubtless often occur.

However this may be, there is an essential difference between game and stock in their relation to these parasites. For while the game is not, so far as is known, in any way inconvenienced even by double infections of the trypanosomes indigenous to its habitat—whether directly or cyclically transmitted—cattle, as a rule, cannot survive even brief contact with the game-tsetses, and are particularly sensitive to strains of *T. congolense* in non-tsetse areas. In localities where game-tsetses are present and game plentiful, a certain percentage of the wild fly will always be found to carry the developmental forms of the proboscis-and-gut group of trypanosomes. In *G. palpalis* areas, however, matters are different. The commonest flagellate found in this fly in nature is probably *T. grayi*. Of the mammalian trypanosomes, the proboscis-only group is most commonly found, represented by *T. vivax* and *T. uniforme*; the gut-and-gland group, usually in the form of *T. gambiense*, is also of common occurrence; but the proboscis-and-gut group is distinguished by the comparative rarity with which it develops in wild *G. palpalis*.

So far as I can determine, three instances are recorded of the occurrence of developmental stages of the proboscis-and-gut group in wild *G. palpalis*. These are as follows:—Warrington Yorke and Blacklock (1915), investigating an epidemic of cattle trypanosomiasis in Sierra Leone, found nineteen out of one hundred and forty-seven cattle infected with trypanosomes; *T. congolense* was found in sixteen, *T. vivax* in seven, and *T. gambiense* in one. In the course of the dissection of four hundred wild *G. palpalis* caught in the same neighbourhood, proboscis-only infections were found in fifteen flies, gut-only in two, and gut-and-proboscis in four.

Macfie (1915) dissected seventy-five wild *G. palpalis* from near Accra, and found eleven infected with flagellates—three gut-and-gland, three proboscis-only, and one proboscis-and-gut. In the review of these experiments, there follows the seemingly contradictory comment that 'none of these infections resembled stages in the development of *T. congolense*.' Possibly the flagellates in the proboscis of the single gut-and-proboscis fly were not fixed, and, in consequence, were not regarded as true proboscis-forms.

The third instance occurred in my own work (1916) in the Northern Province of Uganda, where, in two areas, proboscis-

and-gut infections of wild *G. palpalis* were found, amounting in one case to 1·4 per cent. and in the other to 5·2 per cent. of the flies dissected. Allowing for the possible occurrence in some of these flies of double infections, the last figure is sufficiently high to prove conclusively that, in this area, the *congolense-nanum* type of trypanosome is carried cyclically by *G. palpalis*.

In the face of these examples it is the more remarkable that no instance has hitherto been recorded of the occurrence of this group of trypanosomes in the wild *G. palpalis* of the Victoria Nyanza. At many points along the shores of this great lake, cattle came in contact with this tsetse; and there are long stretches of shore in Busoga where game and *G. pallidipes* occur immediately behind the *G. palpalis* shelter, and where the latter fly has plenty of opportunity for picking up parasites of the *T. congolense* group. *T. vivax*, *T. uniforme*, and a member of the gut-and-gland group are all found in these lakeshore *G. palpalis*. During the fourteen odd years that have elapsed since the depopulation of the Sesse Islands, the Situtunga have multiplied enormously, and the tsetses on these Islands have come to rely on the antelope to a great extent for food. The three trypanosome species just mentioned, are common in these Situtunga, but on no occasion has the *T. congolense* type been found in their blood. Apparently *G. palpalis*, in its natural environment, is not, as a rule, suited to the cyclical transmission of these trypanosomes.

A possible explanation of the absence of the proboscis-and-gut group from the fly on the Victoria Nyanza is, that the strains with which the fly come in contact are directly transmitted strains, which have lost the power of cyclical development in *Glossinae*. But this explanation alone is inadequate to account for the absence of these parasites from this huge fly area.

II. PREVIOUS WORK ON THE TRANSMISSION OF THE PROBOSCIS-AND-GUT GROUP OF TRYPANOSOMES BY *G. PALPALIS*

The Royal Society's Commission in Uganda (1910) experimented with wild and with laboratory-bred flies. Two successful transmissions were reported. In one experiment, with wild *G. palpalis*, the flies became infective to a clean animal twenty-one

days after the first infecting feed; in the other, in which laboratory-bred flies were employed, the flies were infective on the fourteenth day of the experiment. The authors remark that these last experiments were open to fallacy, as an epidemic of trypanosomiasis, due to a member of the 'proboscis-and-gut' group, was occurring at the time in the neighbourhood of the laboratory. I think it probable that the same explanation applies to the wild fly transmission also. No positive flies were found on dissection of the flies in these experiments, and at the time, it was not known that the developmental cycle of *T. congolense* included an invasion of the proboscis of the fly. Subsequent experience has shown it to be improbable that the trypanosomes of this group can complete their cycle in *G. palpalis* in such a short time as twenty-one days.

Of the attempts by the same Commission to transmit *T. nanum* by *G. palpalis*, we read that the only experiment attempted was 'unsatisfactory, as trypanosomes appeared in the first healthy goat a few days after the fly had fed on infected animal,' *i.e.*, it was a natural infection due to some agency outside the experiment.

In view of the ease with which these 'proboscis-and-gut' trypanosomes are propagated in nature by agents other than tsetse-flies, it is, in my opinion, unsafe to carry out experiments at a spot where the disease is already existent, especially if ruminants are employed to demonstrate the transmission.

In 1911, Fraser and Duke carried out seven transmission experiments with laboratory bred flies. One experiment was successful, trypanosomes appearing in the blood of the clean monkey on the eighty-fifth day after the first infecting feed of the flies. On the ninety-sixth day of the experiment a fly died which showed a heavy proboscis-infection. This fly, after infecting the clean monkey, had had access to two other clean monkeys, upon one of which it certainly fed several times. Neither of these last two animals became infected. At the time, it was supposed that the insect must have lost its infectivity, but I now believe that the explanation lay in the different resistance of the host-animals.

In the dissections of these experiments, twenty positive flies were found in a total of four hundred and twenty-seven dissected. Among these were five flies with flagellates established in the proboscis. One of these infected the monkey in the positive

experiment. Two occurred in another experiment, and fed repeatedly on a clean monkey without causing infection; they never had access to a ruminant animal. The remaining two flies with proboscis-infections occurred in a third experiment, and died on the fifty-ninth and seventy-fourth days after the original infecting feed; they both fed repeatedly on a clean monkey without ever infecting it.

The contents of the proboscis of all these five positive flies were injected into rats, without infection resulting. In these experiments no infection of the proboscis was met with in flies dissected before the fiftieth day after the first infecting feed. The salivary glands of these positive flies were all negative to flagellates. In all these experiments the clean animal employed was a monkey. No trypanosome disease existed in the vicinity of the laboratory at the time.

In 1911-12 experiments were carried out on the transmission of *T. nanum* by laboratory-bred *G. palpalis* in Uganda. In the first set of experiments a goat was used for the infecting feeds; one hundred and seventy-three flies were dissected during these experiments; no infected flies were obtained and no transmission occurred.

In the next series of experiments an infected sheep was employed, on which the flies fed much more readily, and the parasite was transmitted to a clean calf. Three hundred and twenty-two flies were dissected, of which twelve were infected, five showing flagellates established in the proboscis. In only one of the proboscis-infections were flagellates seen in the hypopharynx; the labrum of this fly contained great clusters, while a few individuals were present in the hypopharynx. This fly died on the twenty-fifth day after the first infecting feed—the earliest recorded date for the infection of the proboscis of *G. palpalis* by a member of this group of trypanosomes.

In 1911-12 another series of transmission experiments were carried out with *T. congolense* and laboratory-bred *G. palpalis*. The animal used for infecting the flies was a young bushbuck which had been born at the laboratory and, when a few months old, had been infected by syringe inoculation with the Mpumu Laboratory strain. In the course of these experiments, seven hundred and forty-six flies were dissected, of which six hundred and thirteen lived

until the thirtieth day after their first feed on infected blood. Fifteen flies were found to contain flagellates; four had proboscis-infections; and one a heavy infection of the sucking-stomach, with no flagellates in the proboscis. The box which was apparently responsible for the successful transmission, contained this fly with the infected sucking-stomach and none with infected proboscis. The four flies with infected proboscides died respectively on the seventy-sixth, one hundred and fourth, and one hundred and forty-first days after the first infecting feed. A ninetieth day fly showed flagellates in the hind-gut only.

It must be noted that the strain of *T. congolense* kept at the Mpumu Laboratory was derived from cattle, from localities where tsetse-flies are absent or scarce. It is, therefore, probable that it was a directly transmitted strain, before it commenced its career at the laboratory. Too much stress, therefore, cannot be laid upon its behaviour when exposed to tsetses.

The strain of *T. nanum*, on the other hand, came from cattle in the *G. pallidipes* country in Toro Kingdom, and Sheep Experiment 59, by which the flies were infected, was the second passage animal from the original ox.

As already stated, the strain of *T. congolense* used in the experiments now to be set forth is known to be a cyclically carried wild fly strain.

III. HISTORY OF THE TRYPANOSOME STRAIN EMPLOYED IN THE TRANSMISSION EXPERIMENTS PERFORMED AT ENTEBBE DURING 1922 and 1923

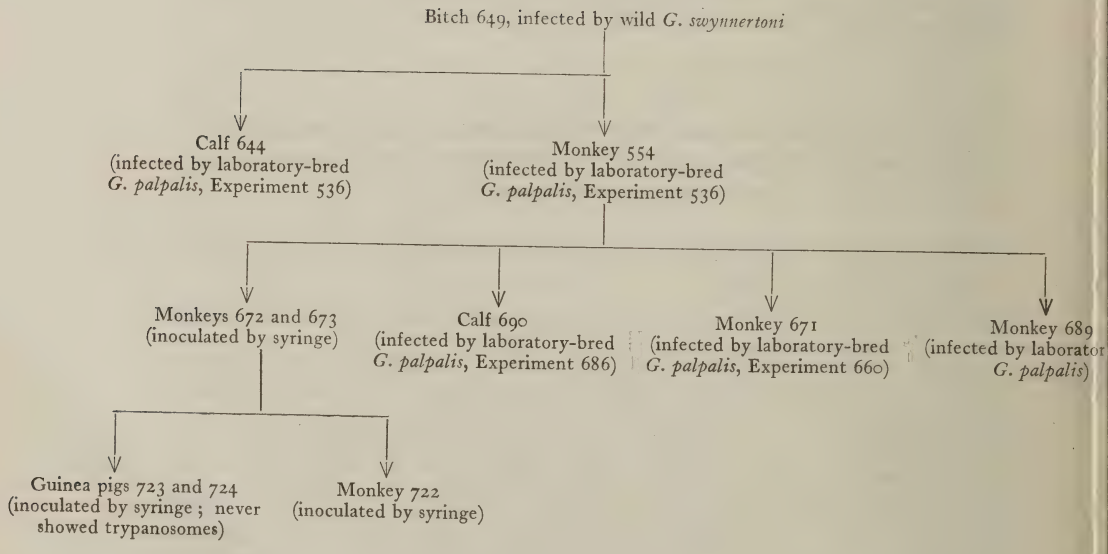
This strain was brought back from Mwanza, in August, 1922, in Bitch Experiment 649.

On August 1st, 1922, Experiment 649 was fed upon by a batch of wild *G. swynnertoni*, of which two, on subsequent dissection, were found to have proboscis-and-gut infections, the salivary glands being negative. On August 8th, 1922, a single trypanosome of the *T. congolense* type was seen in the animal's blood. On August 9th, 10th and 11th, the blood was negative to fresh film examination; on the 12th a few trypanosomes were seen, and thereafter the animal was negative to daily examination of stained thick films

until September 13th. From this date trypanosomes were found at frequent intervals in the course of the daily routine examinations, until February 2nd, 1923. Then followed a series of negative examinations until February 28th, when a few parasites were seen in a stained thick film. Trypanosomes were next seen, on a single occasion only, on August 20th, 1923. For a month or so after arriving at the Entebbe Laboratory, the animal was languid and seemed to be losing condition. In October she showed signs of being pregnant; the mammae enlarged and the animal became fatter, but in a week or two these symptoms disappeared. The general condition was now excellent. She became pregnant about the middle of June, and on August 24th, 1923, at 12 o'clock, two healthy puppies were born, followed, an hour and a half later, by a third which died an hour after birth.

The subsequent upkeep of the strain from this bitch is shown in Table I.

TABLE I.



IV. ANIMAL REACTIONS OF THE STRAIN OF *T. CONGOLENSE* REFERRED TO IN THIS PAPER

The *T. congolense* isolated by Bruce's Commission and employed in their original transmission experiments at Mpumu, lost some of its virulence during two years of maintenance at that laboratory. The average duration of the disease in ten completed monkey experiments carried out by Bruce was sixty-three days; one animal died after one hundred and eighty-one days, the average for the other nine being forty-eight days. Another monkey was alive after two hundred and sixteen days. In eight completed dog experiments the average duration was forty-three days. Of three monkeys inoculated with the same strain eighteen months later, one died after thirty-five days, one after one hundred and fifty days, and the other was still alive after two hundred and three days.

The animal reactions of the strain from the Mwanza Fly Belt are as follows:—

Table II.

	Incubation period, in days	Duration of disease, in days
Monkeys—		
554	78 days or more	127 at least.
671	47 days or more	Still alive after 145 days.
689	?	47th day after first appearance of trypanosomes of Fly Experiment 686.
672	16	Still alive after 188 days.
673	16	64 (trypanosomes swarming in blood).
722	38	43 (trypanosomes swarming in blood).
Calves—		
640	10	Alive and apparently well after 7 months.
644	8	Alive and apparently well after 9 months.
690	23-25	Alive and apparently well after 107 days.

V. TECHNIQUE OBSERVED IN THE CONDUCT OF THESE TRANSMISSION EXPERIMENTS

The transmission experiments were carried out according to the methods pursued for many years in the Uganda Laboratory. The newly hatched flies are placed in wire-sided boxes and fed and starved on alternate days, dead flies being removed for dissection each morning. During the act of feeding, a wet rag covers the top side of the box, while the other side is closely applied to the animal's skin. Feeding continues until the flies have had all the blood they want. At the termination of the experiment the survivors are killed with chloroform vapour and dissected. Experience has proved that the rough and ready method of holding the box in the smoke of a fire, though effective in killing the flies, also kills the flagellates they contain, and makes the identification of light infections more difficult. Throughout the experiments the boxes are kept on stones resting in dishes of clean water.

Whenever a fly with a proboscis-infection was found, the animals upon which the insect had fed were examined daily by means of a stained thick blood-film. Otherwise, all experimental animals were examined daily by careful inspection of fresh unstained blood-films.

VI. THE TRANSMISSION EXPERIMENTS

The actual experiments will now be set forth. In the 'positive-flies' column the contents of the brackets refer to the number of flies with infected proboscides. In the 'remarks' column will be found the result of the experiment, positive or negative, according as the clean animals develop trypanosomes or not.

(A) Experiments in which some of the flies developed proboscis-infections.

(1) Experiments in which cyclical transmission of *T. congolense* from sick to healthy animal occurred.

Table III.

Experiment 536.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1922 Sept. 25-27	1-3	Flies fed on bitch 649 ...	...	*T. congolense* not seen in stained thick films of bitch's blood.
,, 28	4	Starved	...	
,, 29-Nov. 21	5-58	Fed on clean monkey 554	2 (1 proboscis +)	*T. congolense* first seen in 554's blood on 13 Feb., 1923.
Nov. 22	59	Starved	...	
,, 23-Dec. 1	60-68	Fed on clean calf 644 and on 554 on alternate days	...	*T. congolense* first seen in calf's blood on Dec. 1.
Dec. 2	69	Starved	...	
,, 3-4	70-71	Fed on clean monkey 611	...	Monkey 611 never showed trypanosomes.
,, 5	72	Remaining 28 flies dissected	3 (1 proboscis +)	

Flies dying before 16th day were ignored.
Total number of flies dissected during the experiment = 68.
Number alive on 25th day of the experiment = 64.

Remarks. November 27th was the last date on which the flies had access to 554, the incubation period in the monkey was thus very long, amounting to at least seventy-eight days. In contrast to this the incubation period in the calf was only nine days.

TABLE IV.

EXPERIMENT 660.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923				
Feb. 16	1	Flies fed on monkey 554	...	*T. congolense* present in 554's blood.
,, 17	2	Starved	...	
,, 18-19	3-4	Fed on monkey 554 ...	...	*T. congolense* present in 554's blood.
,, 20	5	Starved	...	
,, 21-23	6-8	Fed on monkey 554 ...	...	*T. congolense* present in 554's blood.
,, 24	9	Starved	...	
,, 25-April 8	10-52	Fed on clean monkey 671 on alternate days	1 (proboscis +++) 51st day	Monkey 671 first showed *T. congolense* on 24 May, 1923
April 9-10	53-54	Starved	...	
,, 11	55	Remaining 19 flies dissected	2	

Flies dying before the 22nd day of the experiment were ignored.
Total number of flies dissected = 58.
Number alive on 25th day of experiment = 55.

Remarks. Note the long incubation period of *T. congolense* in monkey 671, *i.e.*, from, say, the first week in April until 24th of May.

Table V.

Experiment 686.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923				
Mar. 11-13	1-3	Flies fed on monkey 554	...	*T. congolense* present in 554's blood.
,, 14-15	4-5	Starved	...	
,, 16-May 13	6-64	Fed on clean monkey ...	1 (proboscis +) 54th day	Monkey 689 first showed *T. congolense* in blood on 23 May, 1923.
May 14	65	Starved	...	
,, 15-17	66-68	Fed on clean calf 690 ...	...	Calf 690 first showed *T. congolense* in blood on 7 June, 1923.
,, 18-19	69-70	Starved	1 (proboscis +) 69th day	
,, 20	71	Fed on 689	...	
,, 21	72	Starved	...	
,, 22	73	Fed on 689	...	
,, 23-24	74-75	Starved	...	
,, 25	76	Remaining 14 flies dissected	...	

Flies dying before 12th day were ignored.
Total number of flies dissected = 49.
Number alive at 25th day = 46.

Remarks. It is impossible to estimate the incubation period in monkey 689. In the calf, trypanosomes appeared twenty-three to twenty-five days after the infecting feed.

(2) Experiments in which no transmission occurred.

TABLE VI.

EXPERIMENTS 597-8.

Date	Day of Experiment	Procedure	Positive Flies found	Remarks
1922 Nov. 18	1	Flies fed on dog 649 ...	...	*T. congolense* not seen in dog's blood.
,, 19	2	Starved	...	
,, 20-21	3-4	Fed on dog	...	*T. congolense* not seen in dog's blood.
,, 22	5	Starved	...	
,, 23-Dec. 25	6-38	Fed alternate days on clean monkey 602	...	Monkey 602 never showed trypanosomes: examined by thick stained films till 15 June, 1923.
Dec. 26	39	Starved	...	
,, 27	40	Fed on clean dog *X* ...	...	Dog *X* never showed trypanosomes.
,, 28	41	Starved	...	
,, 29	42	Fed on dog *X*	...	
,, 30	43	Starved	2 (1 proboscis +)	This fly had fed on dog *X*.
1923 ,, 31-Jan. 16	44-60	Fed on alternate days on monkey 602 and dog *X*	...	
Jan. 17	61	Remaining 32 flies dissected	...	

Flies dying before the 11th day of the experiment were ignored.
Total number of flies dissected = 113.
Number alive at 25th day of experiment = 105.

Remarks. Neither of the clean animals of this experiment became infected. The infection of the proboscis in the forty-three day fly was not heavy, and may have been established after the fly had been removed from contact with monkey 602.

Possibly, in the earlier stages of the invasion of the proboscis, the fly is only capable of infecting very susceptible mammals, on account of the small number of trypanosomes which it inoculates.

TABLE VII.

EXPERIMENT 658.

Date	Day of Experiment	Procedure	Positive flies found	Remarks
1923				
Feb. 15-16	1-2	Fed on monkey 554 ...	...	*T. congolense* present in 554's blood.
„ 17	3	Starved	...	
„ 18	4	Fed on monkey 554 ...	...	T. *congolense* present in 554's blood.
„ 19	5	Starved	...	
„ 20	6	Fed on monkey 554 ...	...	*T. congolense* present in 554's blood.
„ 21	7	Starved	...	
„ 22	8	Fed on monkey 554 ...	...	*T. congolense* present in 554's blood.
„ 23	9	Starved	...	
„ 24-April 21	10-66	Fed on clean monkey 668	1 (proboscis +) 66th day	Monkey never showed trypanosomes: examined daily, stained thick films till 23 July, 1923.
April 22	67	Starved	...	
„ 23-25	68-70	Dog ♀	...	Dog never showed trypanosomes.
„ 26	71	Starved	...	
„ 27-May 1	72-76	Fed on monkey 668 and starved alternate days	...	
May 2-3	77-78	Fed on clean calf ...	...	Calf never showed trypanosomes.
„ 4-6	79-81	Starved	...	
„ 7	82	Remaining 12 flies dissected	...	

Flies dying before the 17th day were ignored.
Total number of flies dissected = 41.
Number alive on 25th day = 47.

Remarks. The only positive fly of this experiment fed repeatedly on monkey 668 without causing infection. Unfortunately this fly died before feeding on the dog or the calf.

(*B*) *Experiments in which no flies with infected proboscides were found.*

(In none of these experiments did transmission occur.)

Table VIII gives a summary of these experiments.

Table VIII.

Experiment	Date of infecting feeds	Infecting animals	Number of Flies			Duration of Experiment in days	Day of Experiment on which dissection began
			Alive 25th day	Dissected during Experiment	Containing flagellates in gut		
520	19-21.9.22 ...	Bitch 649 ...	59	63	0	77	18th
600-1	22-24.11.22 ...	Bitch 649 ...	85	123	0	57	13th
643	9, 10 and 12.2.23	Calf 644 ...	58	66	0	44	16th
662	18-21.2.23 ...	Calf 644 ...	40	54	2	68	13th
665	21-23.2.23 ...	Calf 644 ...	37	37	0	65	24th
664	19-21 and 23.2.23	Monkey 544 ...	46	50	0	77	18th
667	23-25.2.23 ...	Calf 644 ...	53	63	0	61	11th
674	1-3.3.23 ...	Calf 644 ...	39	46	0	74	15th
678	3-6.3.23 ...	Calf 644 ...	46	47	0	72	19th
684	8-10.3.23 ...	Monkey 554 ...	29	31	0	78	16th
693	15-17.3.23 ...	Monkey 673 ...	50	53	1	83	19th
700	19-22.3.23 ...	Monkey 554 ...	44	53	0	82	14th
			586	686	3		

The results of dissection of the positive flies of all the above experiments are shown in Table IX.

Table IX.

Experiment	Number of fly	Day of dissection reckoned from first infection feed	Distribution of flagellates		Remarks
			Gut	Proboscis	
693	1	20th day	++	o	
662	2	21st day	+++	o	
536	3	26th day	+++	o	
543	4	34th day	+++	o	
579-8	5	43rd day	+++	o	
...	6	43rd day	+++	+	
660	7	51st day	+++	+++	
543	8	52nd day	+++	+++	
662	9	53rd day	+++	o	
686	10	54th day	+++	++	
660	11	55th day	+++	o	
...	12	55th day	+++	o	
536	13	57th day	+++	+++	Sucking stomach +++
642	14	58th day	+++	o	
536	15	62nd day	+++	o	
658	16	66th day	+++	+++	
686	17	69th day	+++	++	
536	18	72nd day	+++	o	
...	19	72nd day	+++	+++	
...	20	72nd day	+++	+++	

VII. DISCUSSION OF THE EXPERIMENTS

It will be seen from Table IX that the earliest date at which flagellates were found in the proboscis was in a forty-three day fly. It is, however, possible that the invasion of the proboscis in some of the older flies took place earlier than this. On the other hand,

several infected flies lived considerably longer than forty-three days without the flagellates reaching the ' anterior station.'

The experiments of Group A show that flies with heavily infected proboscides may feed upon a clean animal without causing infection. It is interesting to note that, when this happened, in each case the clean animal was either a monkey or a dog: whenever a calf was bitten by a fly with an infected proboscis, the animal developed trypanosomes.

It was hoped, by exposing, alternately, different species of clean animals to the infected flies of these experiments, to throw light upon the biological significance of the differences in virulence and of host proclivities shown by the trypanosomes of this group. But, unfortunately, on several occasions the infected fly died before the box was transferred to a second, or a third clean animal. It was thus not possible to ascertain whether a fly may be infective to a ruminant and yet be unable cyclically to infect a monkey or a dog.

The strain of *T. congolense* used in these experiments was of comparatively low virulence. On several occasions the incubation period in monkeys, before trypanosomes appeared in the peripheral blood, was very long. In the case of monkeys Nos. 554 and 671, both of which were infected by flies, the incubation periods were seventy-eight and forty-seven days, respectively; with monkey Experiment 722, infected by the syringe, thirty-seven days elapsed before trypanosomes were detected. The incubation periods in the calves infected by flies were nine and twenty-three to twenty-five days. Similarly, in my experiments at Mpumu, the incubation period in the only monkey infected by cyclical transmission was thirty-three days, while in another monkey, infected by means of the syringe, the incubation period was forty-seven days.

In contrast to this, the average incubation period in thirteen monkeys inoculated by Bruce (1910) in Uganda was 12·3 days, maximum twenty-one days. Bruce was employing a virulent strain—probably directly transmitted—derived from a cattle epidemic. This strain was maintained for upwards of two years by syringe inoculation from monkey to monkey before being subjected to the transmission-experiments just referred to.

Both the *nanum* and the *congolense*-forms of this group—distinguished from one another by their different behaviour in

monkeys and dogs—have been recovered from wild game tsetses, the *nanum*-form being the most common. The *congolense*-form is acknowledged to be the more virulent, and is almost always present in cattle epidemics due to this group of trypanosomes. The evidence supplied by the experiments set forth in Section 6, taken in conjunction with the observations already recorded on this group of trypanosomes, suggests that cyclical transmission of *T. congolense* tends to the acquisition by the trypanosome of a low degree of virulence, which may be associated with inability to infect such animals as monkeys and dogs in nature. Directly transmitted natural strains, on the other hand, usually possess greater virulence, and readily infect these two animals.

SUMMARY

1. Three out of seventeen cyclical transmission experiments, performed with laboratory-bred *G. palpalis* and a wild fly strain of *T. congolense*, were successful. In the course of these seventeen experiments, one thousand and fifteen flies were dissected, of which eight hundred and ninety-three lived until the twenty-fifth day after their first feed on an infected animal; eight of these flies had flagellates established in the proboscis.

2. In all the successful transmission experiments, one or more flies with proboscis-and-gut infections had fed upon the clean animals which acquired infection. There is every reason to believe that these flies were responsible for the transmission.

3. Flies with equally intense proboscis-and-gut infections were found in two experiments in which no transmission occurred. Several of these flies had fed repeatedly on clean monkeys without infecting the animals; in no case, however, did a fly with a proboscis-and-gut infection feed upon a clean calf without infecting it. It would appear, therefore, that monkeys are less susceptible to this strain of *T. congolense*, carried by *G. palpalis*, than are calves.

4. In several cases the incubation period in the monkey was very prolonged.

5. The strain of *T. congolense* used in the experiments is less virulent than the [strain] used by Bruce at Mpumu, in Uganda.

The Mpumu strain was almost certainly directly transmitted before its arrival at the laboratory, while the strain here described was carried cyclically by wild tsetse. It is possible that there is a definite relation between the virulence of a strain and its method of transmission.

6. The apparent fact that the wild *G. palpalis* of the Uganda shores of Victoria Nyanza do not carry trypanosomes of the proboscis-and-gut group is to be explained, in part at any rate, by the partiality of the fly for animals which are not susceptible to this group of trypanosomes. It would appear, however, from the experiments above described, that this *G. palpalis* is less fitted to act as a true intermediate host of the *T. congolense* group of trypanosomes than of *T. vivax*, *T. uniforme*, and *T. brucei*.

My thanks are due to Dr. Mary Martin, Assistant Bacteriologist, Uganda Protectorate, for valuable help in the conduct of these experiments.

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ON A NEW SPECIES OF *PHLEBOTOMUS* FROM JAPAN

BY

R. NEWSTEAD, F.R.S.

(*Received for publication* 15 *October*, 1923)

Phlebotomus squamirostris, n. sp.

Superior claspers of the male with two pairs of stout spathuliform spines, the proximal pair arising from a well-developed tubercle. Terminal segment of the palpi slightly longer than the fourth. Rostrum (labium) densely scaly.

Male. Abdominal hairs recumbent and uniformly pale ochraceous as on other parts of the body. Wings faintly iridescent; costal hairs scarcely darker than the rest. Rostrum (labium) (fig. 1 *e*) densely clothed with long, forwardly directed, non-deciduous scales. Antennae with relatively long segments, and short, unilateral, geniculated spines; third segment (fig. 1 *a*) projecting beyond the tip of the proboscis to a distance of 0·1 mm. or more, the geniculated spine placed near the distal fourth. The spine on the other segments is placed proximally a little in advance of the articulation (fig. 1 *b*). Palpi rather robust; the third segment distinctly incrassate, the fifth about one-third longer than the fourth; formula 1 (2, 4), 3, 5. Wings (fig. 1 *c*) lanceolate; the anterior branch of the second long vein about equal in length to the distance between the forks. Armature (fig. 1 *d*); superior claspers with two pairs of stout spathuliform spines, arranged in two pairs; the first attached to a well-marked tubercle slightly beyond the middle of the segment; the second pair terminal, each arising from a tubercle. Inferior claspers relatively short, and either equal to, or very slightly longer than the proximal segment of the superior claspers.

Length: 2·7 mm.; length of wing: 1·6 mm.; antenna: 2·3 mm.

Female. Arrangement of abdominal hairs and colour as in the male; but the body is slightly more robust.

JAPAN:—Agori: July 19th, 1916, 1 ♂ (*Dr. Shinichiro Yamada*); Matzuyama: June 25th, 1916, 1 ♂, 1 ♀ (*Dr. S. Komatsu*).

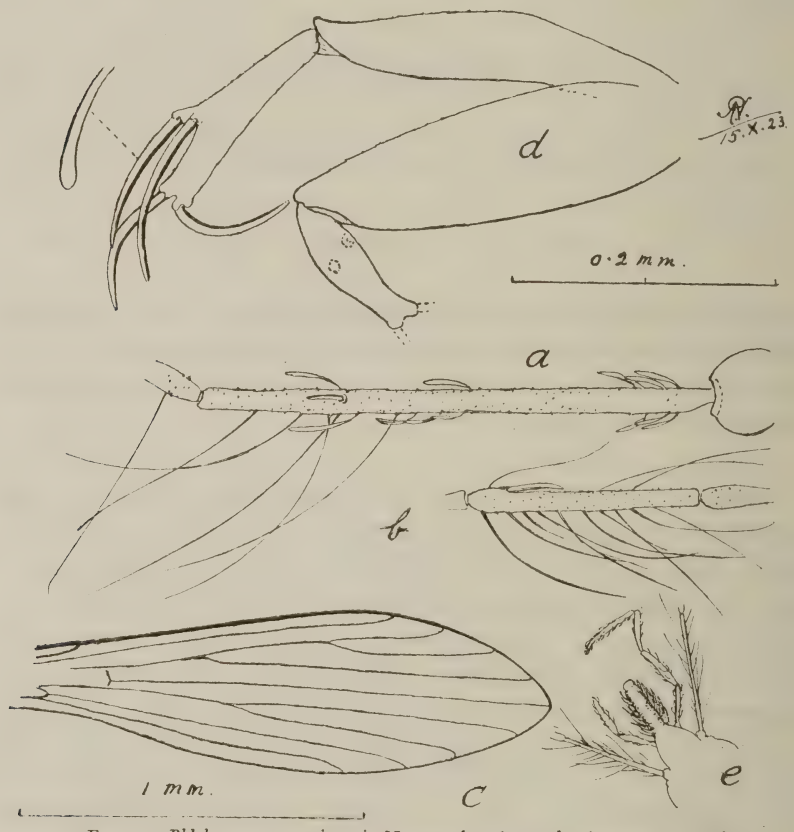

FIG. 1. *Phlebotomus squamirostris*, Newstead. ♂. *a*, *b*—Antenna; *c*—Wing; *d*—Armature; *e*—Rostrum. *a*, *b* and *d* to same magnification.

I am indebted to Dr. S. Yamada, Institute of Infectious Diseases, Imperial University of Tokyo, Japan, for the opportunity of studying this material.

GLOSSINA ZIEMANNI, GRÜNBERG, A SYNONYM OF *GLOSSINA PALPALIS* SUB-SPECIES *FUSCIPES*, NEWSTEAD

BY

R. NEWSTEAD, F.R.S.

(*Received for publication* 15 *October*, 1923)

In April, 1912, Dr. K. Grünberg* described the tsetse-fly, *Glossina ziemanni*, which he considered distinguishable from all other known species by its uniformly dark colouration, its heavily infuscated wings and its entirely black tarsi. Furthermore, by the metallic sheen or iridescence of the scutellum and parts of the abdomen; and by the presence also of a dirty ashen grey pollinose covering. His description was based upon 1 ♂ and 5 ♀♀ (preserved in alcohol), which were taken at Mina, Mbam R., Cameroons, in 1912, and forwarded to Berlin by Dr. H. Ziemann.

Being desirous of examining examples of the species in question, I applied to Dr. G. Enderlein, of the Berlin Zoological Museum, for the loan of specimens of both sexes; this he very willingly granted, and at the same time forwarded the type ♂ and ♀ to this School.

On making an examination of the external characters, one saw at once that the remarkable iridescent colouration, the 'pollinose' covering and the deep blackish brown tinge of the wings were clearly caused by impurities in the alcoholic preservative; these impurities had so completely masked the true colours and pattern that it was quite impossible to determine the species without making a microscopical examination of the armature.

Having dissected out the structures—a task of great difficulty owing to the intense hardening of the integument—one is now able to say, quite definitely, that the morphological characters are specifically identical with those of *Glossina palpalis* sub-species *fuscipes*, Newst.

* Eine neue Tsetse-fliege aus Kamerun : *Sitzgsber. Ges. Naturf. zu Berlin.* Jahrg. 1912, No. 4, p. 246 (April, 1912).

One may add that the presence of foreign matter on the wings is easily demonstrable by passing a beam of light through the membrane, when almost the entire surface can be seen to be more or less covered by dark granular bodies which, in places, have blotted out the true character of the membrane and have also matted the fine hairs together in many parts.

One regrets exceedingly to have to relegate Dr. Grünberg's species to the synonymy of *Glossina palpalis* sub-species *fuscipes*, Newst., but the study of the taxonomic characters of his material affords convincing proof that *G. ziemanni* must sink.

We tender our sincere thanks to Dr. Enderlein for his great kindness and courteous assistance, without which it would have been quite impossible to clear up the synonymy.

MALIGNANT GROWTHS IN NATIVES OF SIERRA LEONE

BY

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AND

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(*From the Sir Alfred Lewis Jones Research Laboratory*)

(*Received for publication* 1 *November*, 1923)

It is impossible to form an opinion on the frequency of malignant growths in natives of West Africa, as the aborigines seldom consult medical men. Nevertheless, it has often been stated that malignant growths are rare or absent in West Africans, and this has been attributed to non-adoption of European habits. As a proof of the alleged relationship between civilised habits and malignant growths, it has been stated that such growths have only been recorded among Creoles who have more or less adopted European habits, and in those living in coastal towns where European influence is active. Thus Renner (1910) states that cancerous and other malignant growths have been increasing among the Creoles of Sierra Leone in recent years, but that they are rare or absent in aborigines. He further states, that although the Fantis of the Gold Coast have been in contact with Europeans for centuries, malignant growths are rare or absent among them, because they have resisted the inroads of European civilisation. Macfie (1922), on the other hand, writing of the prevalent diseases of the Gold Coast, states:—'Tumours are probably as common as elsewhere, but sarcomas appear to be rather commoner and carcinomas are said to be rare, a belief which may be due to the fact that the hospital clientèle represents only a small and selected portion of the total sick.' Dyce Sharpe (1923) states that carcinomas are rare, differing in this respect from sarcomas, even among the population of the coastal towns of West Africa. Cameron Blair (1923) states that he has never seen a case of carcinoma or

sarcoma in twenty-two years in Nigeria, and that the occasional carcinomas found by medical men in the coastal regions, occur chiefly in natives who have come in contact with Europeans. It must be borne in mind, however, that Europeanisation, whether or not it were conducive to the spread of malignant growths, would certainly be responsible for intelligent natives afflicted with them consulting medical officers. Thus civilisation may be wrongly blamed for the spread of the disease, when it is only responsible for its diagnosis.

In view of the alleged rarity of malignant growths in West African natives, the following record of seven cases, five of which came under our personal observation between May and November, 1922, may be of interest. Five of these cases occurred in aborigines and two in Creoles.

CASE 1. An aborigine (male Timne, aged 40 circ.) gave a history of an ulcer on the plantar surface of the right foot following an injury. When seen by us there was a fungating growth from the base of a chronic ulcer, which on section proved to be a melanotic sarcoma. There was a large secondary growth in the right groin.

CASE 2. An aborigine (male Timne, aged 60 circ.) had a painful growth on the scrotum which was found on section to be an epithelioma.

CASE 3. A Creole (male, aged 58) had an ulcer which commenced on the upper lip. Sections showed the ulcer to be an epithelioma. There were secondaries in the glands of the neck on both sides.

CASE 4. An aborigine (male Timne, aged 50 circ.) had a tumour on the right side in the temporal region, exopthalmos of the right eye and complete hemiplegia on the left side. *Post-mortem:* Meningo-sarcoma which had destroyed a large area of bone on the right side, involving the temporal, frontal parietal and sphenoidal bones, infiltrated the muscles and subcutaneous tissue, and also invaded the right orbit. There were large secondaries in the liver.

CASE 5. An aborigine (male Mandingo, aged 28 circ.) died in the Colonial Hospital, Freetown. Dr. J. D. Dimock, W.A.M.S., found a tumour in the liver, which he kindly presented to the Sir A. L. Jones Research Laboratory. Sections of the tumour showed it to be primary carcinoma of the liver.

CASE 6. A Creole (female, aged 42) complained of debility. *Post-mortem:* Tumour of the liver, which on section proved to be a primary carcinoma.

CASE 7. Dr. C. H. Allan, W.A.M.S., sent a piece of liver containing growth which he obtained from a post-mortem on an aborigine (Sherbro). Sections showed the liver to be invaded by a secondary carcinoma.

In addition to the above material, we examined a tumour of the breast which Capt. M. Jackson, W.A.M.S., removed from a Mende woman, aged 50 (circ.). The tumour on section proved to be a carcinoma.

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A PRELIMINARY ACCOUNT OF THE RESULTS OF SURVEYS FOR BREEDING-PLACES OF MOSQUITOES IN NORTH WALES

BY

W. REES WRIGHT, B.Sc.

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INTRODUCTION

The present paper is an account of the results of an investigation into the biology of the species of CULICIDAE, or mosquitoes, present in various parts of North Wales. The work, which was carried out under the Department of Zoology and the Agricultural Zoology Laboratory of the Department of Agriculture, at University College, Bangor, was begun in October, 1920, and has since been prosecuted whenever circumstances have permitted; that portion of it chiefly dealt with here, viz., observations on breeding-habits, was carried out during the summers of 1921, 1922, and 1923.

In the summer of 1921, my attention was entirely confined to an area of CARNARVONSHIRE bounded by the MENAI STRAITS, the OGWEN RIVER, the SNOWDONIAN MOUNTAINS, and the GWYRFAI RIVER. The elevation of the area worked varies from a few feet above sea-level to about 480 feet at LAKE CWELLYN. The major part of the district is well above the 200-feet contour; the only low-lying land is along the shore of FORYD BAY, an arm of the Menai Straits. In this part there are a relatively large number of slow running ditches and brackish pools. In the rest of the area there are few ponds; drainage is largely by swiftly running natural streams.

The fields are for the most part bounded by stone walls without ditches. The area is moderately wooded; the preponderating trees are oaks and conifers; such tree-hole examination as has been done has yielded negative results (Blacklock and Carter (1920)).

In the summer of 1922, and again in 1923, I re-surveyed the North Carnarvonshire area, while I examined, in addition, streams and ground collections of water in the neighbourhoods of DOLGELLEY and DINAS MAWDDWY, MERIONETHSHIRE, and ABERFFRAW, ANGLESEY (this last in 1922 only).

In all, ten species of mosquitoes have been taken in the larval stage; viz., *Anopheles maculipennis*, *A. bifurcatus*, *Aedes* (*Ochlerotatus*) *detritus*, *A.* (*O.*) *caspius*, *A.* (*O.*) *punctor* var. *meigenanus*, *A.* (*O.*) *rusticus*, *Aedes* (*Ecculex*) *vexans*, *Theobaldia annulata*, *Theobaldia* (*Culicella*) *morsitans*, and *Culex pipiens*. The first three and last four have also been obtained as imagines. The nomenclature employed is that of F. W. Edwards (1921); since this differs from that employed previously (*e.g.*, Lang (1920)), I have thought it advisable to give both the authority for the name, and also, when Lang's specific or generic name differs from that of Edwards, that employed by the former author.

It may be mentioned that the weather varied during the period which this paper covers, from being very dry and warm (1921) to cold and wet (1923).

I have very great pleasure in taking this opportunity of acknowledging my indebtedness to Professor P. J. White, F.R.S.E., Department of Zoology, and Mr. C. L. Walton, Adviser in Agricultural Zoology at Bangor, for much valuable advice, assistance and encouragement; to Professor Warrington Yorke, Professor R. Newstead, F.R.S., and Miss A. M. Evans, of the Liverpool School of Tropical Medicine for valuable assistance in the matter of literature; to Professor Newstead, for helpful criticism also; to Mr. F. W. Edwards, of the British Museum (Natural History), for information as to literature, and for the identification of imagines of *Theobaldia morsitans*; and finally to numerous landowners and farmers, especially the Trustees of the Vaynol Estate and Mr. W. H. Jones, Plas Llanfaglan, Carnarvon, for affording me every facility for the 'field-work' involved in these investigations.

Anopheles maculipennis, Meig.

Anopheles maculipennis, Meigen. Syst. Beschr., Vol I, p. 11 (1818).

This species is everywhere common. In my experience, it will

breed in any moderately clean water containing vegetation. The presence of a moderate amount of current does not affect it; it will breed in swiftly running streams, provided there is enough vegetation to shield the larvae from the full force of the current. As for volume of water, it needs but little. In the laboratory, the larvae may be bred in shallow vessels, offering a large surface to the air, with low mortality; when the surface is small relative to the volume, there is considerable mortality, even if the water be daily artificially aerated.

According to Martini (1921), the Sanitary Staff of the German Army in Macedonia found that this species would not breed in water thickly covered with duckweed. I have obtained larvae from pools thickly covered with *Lemna minor*. I have also obtained larvae from water 'choked' with *Elodea canadense*.

It is a well-known fact that in the later years of last century, and the earlier years of this, there was a continued decrease in the amount of endemic malaria ('ague') in Great Britain and also in other parts of Northern Europe (Wesenburg-Lund, 1921). Various theories have been put forward to account for this, the latest being that independently proposed by Wesenburg-Lund and Roubaud, that a change has taken place in the mode of nutrition of *Anopheles maculipennis*. It has been suggested by Nuttall (Lang, 1918) that unfavourable breeding seasons, by temporarily exterminating *Anopheles* spp. in the ague zones, may have broken the 'chain of infection,' and thus brought about the observed disappearance of the disease; he suggests that very wet seasons may bring this extermination about by washing-out larvae from the localities in which they normally breed. I doubt if this cause would operate in such country as is found in North Carnarvonshire, where there are few ditches, the majority of the Anopheline breeding in isolated pools, etc., not liable to 'wash-outs.' Ague was formerly endemic in parts of the North Carnarvonshire littoral; I have met several farmers who remember a time when it was often difficult to conduct agricultural operations owing to ague among the farm labourers. At the present time, there are thousands of Anophelines in the area, and many imported cases of malaria, but so far as I am aware, there have been no locally contracted cases of malaria within the last few years.

The observations of Macgregor (1921) and James (1922) in

Surrey, and my own in North Carnarvonshire, suggest that a dry season is not likely greatly to affect the Anopheline mosquitoes.

This species generally breeds in the least shaded situations, so that in pools or streams it may be expected on the North side, unless this be shaded, deep, or without vegetation. I have occasionally obtained it from quite shady spots.

On 13th September, 1922, I obtained larvae of this species from a rainwater tank at PLAS LLANFAGLAN, near Carnarvon.

Larvae in all instar, and pupae, may be obtained throughout the summer.

The females hibernate in the warmer farm buildings.

Anopheles bifurcatus (Linn.).

Culex bifurcatus, Linnaeus. Syst. Nat., Ed. X, p. 603 (1758).

This species is the commonest mosquito in North Carnarvonshire. It breeds in the same types of water as does *A. maculipennis*, and also in extremely foul pools in marshes. I have often obtained larvae of the two species together. This species may be bred in the laboratory under the same conditions as *A. maculipennis*.

Feytaud and Gendre (1919) state that, while *Anopheles maculipennis* 'develops above all in stagnant water, clean and sunny (clear pools, lagunes, marshes, etc.), with abundant vegetation, variable temperature,' etc., '*Anopheles bifurcatus* likes pure water . . . cold, with little vegetation. We especially see it in fresh springs, streams through woods . . . wells.' The results obtained by Boyd (1922) and myself are not in agreement with this; Boyd remarks that 'the dyke in which the greatest number (of larvae) were found has the banks overgrown with weeds, and is almost stagnant in parts. The bottom is covered with leaves and decaying vegetation, into which the larvae appear to burrow at times.'

Larvae may be found throughout the year in all instar, and pupae throughout the summer.

The larvae of both species of Anophelines commonly bear a greater or less number of ectozoic Ciliates, very similar in appearance to *Vorticella*. These occur but rarely on Culicine larvae.

The larva hibernates.

Theobaldia annulata (Schrank).

Culex annulatus, Schrank. Beit. Z. Naturg., p. 97 (1776).

This, the largest and most ornamented of our mosquitoes, is common everywhere.

I have never found the larvae except in clean currentless water, but it has elsewhere been found breeding in foul rainwater (R. Newstead). It may be found in both natural and domestic collections.

Normally, this species hibernates as the adult female, in cellars and lofts.

Larvae of this species hibernated, in the winter of 1921-22, in a tank at UNIVERSITY COLLEGE, BANGOR, attaining the adult condition at the end of March, 1922. During the winter the water in the tank was once frozen almost—if not quite—solid, while it was frozen on the surface only, on two other occasions. Boyd (1922) has made similar observations.

Wesenburg-Lund (1921) states that in Denmark this species is exclusively domestic.

Theobaldia (*Culicella*) *morsitans* (Theo.).

Culex morsitans, Theobald. Mon. Cul., Vol. II, p. 5 (1901).

Culicella morsitans (Theo.). Lang. Handbook, p. 102 (1920).

I have obtained this species from one locality only, in a wood near GLAN-RHYD FARM, PENTIR, near Carnarvon (altitude slightly over 350 feet), where I found numerous first and second instar larvae in pools in a ditch, partly filled with fallen leaves and similar debris, on 21st September, 1922. I visited this pool on several later occasions. During the winter, fourth instar larvae were found; these pupated during May and June, 1923. Imagines bred in the laboratory from larvae were sent to Mr. F. W. Edwards, and identified as this species. After May, no larvae of this species were found until 11th October, when I obtained a few. The ditch was full of water most of the time.

According to Wesenburg-Lund (1921), the eggs are deposited on dry earth during July and August. These hatch out in September, the larvae proceeding to the fourth instar during the period 1st October to 1st December. The winter is passed as fourth instar larvae.

Aedes (*Ochlerotatus*) *detritus* (Hal.).

Culex detritus, Haliday. Entom. Mag., Vol. I, p. 151 (1833).

Ochlerotatus detritus (Hal.). Lang. Handbook, p. 89 (1920).

This species has been obtained from the low-lying part of the Parish of LLANFAGLAN, bordering on FORYD BAY, and also from similar land near ABER, Bangor, in late August, 1923.

The larvae occur both among vegetation and in open water; in pools the large fourth instar larvae may often be seen in the clear water towards the centre. Usually it is found in brackish or salty water, but I have taken it from inappreciably saline water and also from slowly running fresh water. In the laboratory, the larvae are easy to breed; they can be kept in shallow dishes filled with water from their original breeding-place, fresh water being occasionally added to compensate for evaporation. I have kept larvae in two cubic centimetres of water apiece, with no losses. By gradually diluting the brackish water, the larvae may become accustomed to, and thrive in, fresh water.

Under laboratory conditions, larvae pupated at various times between 11 a.m. and 6 p.m. on 12th July, 1922, the first imago emerging about 4 p.m. on 16th July. The maximum temperature in the interval was 66° F., the minimum 58° F.

On 29th June, 1923, I placed some dry mud from a ditch in which this species had bred the previous year, in a large dish of water. On 5th July, I noticed a larva swimming in the dish. This cast its skin on the following day, and pupated on the 11th. On the 14th, a female emerged from the pupa. The mean temperature was slightly over 60° F.

The imagines spend the day in the vegetation, around the breeding-places; they may often be beaten out in large numbers. The females are vicious biters, both in nature and under laboratory conditions; the swelling after the bite is, in my experience, more painful than that of any other of our North Wales species. In the laboratory, the females will attempt to feed shortly after emerging from the pupae, before the chitin of the mouth-parts has become rigid enough to allow of piercing the skin.

According to Lang (1920), there are 'at least two generations in the year.' In 1922, I believe there were three in this area. In

late June and early July, and again in late August and early September, larvae were abundant; in the interim there were none. The major part of the second brood had attained the last larval instar at least by 13th September, 1922, when I found only fourth instar larvae and pupae. On 25th November, 1922, I visited several of the pools where I had found larvae during the summer, with the object of obtaining data regarding the hibernation of this species. All pools and ditches were covered with ice; on breaking this and dipping near the margin, I everywhere obtained numerous larvae. All four instar were taken, the first two predominating. This suggests that in the interval between my two visits (13th September and 25th November) the females of the second brood—*i.e.*, females hatched from the larvae and pupae of August-September—had oviposited and that from the eggs emerged the larvae found on 25th November, these being a third generation. Larvae in all instar continued to abound until June, 1923, when they disappeared. In July the pools and ditches dried up. In August they again filled, and larvae were found until 2nd October, 1923. On 31st October no larvae could be found anywhere after a careful search. James (1922) records that larvae were found throughout the year.

According to Wesenburg-Lund (1921) and Lang (1920), this species hibernates as the egg; apparently it can hibernate also as the larva. It survives drought as the egg.

Aedes (*Ochlerotatus*) *punctor* (Kirby) var. *meigenanus*, Dyar.

Culex punctor, Kirby. Fauna Boreali-Amer., Zool. Ins., p. 305 (1829).

Aedes meigenanus, Dyar. *Insecutor Inscitiae Mens.*, Vol. IX, p. 72 (1921).

Ochlerotatus nemorosus (Theobald). Lang. Handbook, p. 91 (1920).

I have obtained larvae of this species from pools in a marsh near LLANELLTYD BRIDGE, DOLGELLEY (altitude well under 50 feet), in company with larvae of *Culex pipiens*, 27th July, 1922; and also nearer DOLGELLEY in company with *Aedes vexans*, 27th July, 1923.

Aedes (*Ochlerotatus*) *caspius* (Pallas).

Culex caspius, Pallas. Reise versch. Prov. Russ. Reich, Vol. I, p. 475 (1771).

Ochlerotatus caspius (Pallas). Lang. Handbook, p. 81 (1920).

I obtained larvae of this species in a pool on FAIRBOURNE (South of Barmouth, Merionethshire) Golf Links, 25th July, 1922; and with *Aedes vexans*, near DOLGELLEY, 27th July, 1923. This species has previously been recorded from Merionethshire (Tal-y-bont, North of Barmouth, see Lang, 1920) by Mr. F. W. Edwards.

Aedes (*Ochlerotatus*) *rusticus* (Rossi).

Culex rusticus, Rossi. Fauna Etrusca, Vol. II, p. 333 (1790).

Ochlerotatus rusticus (Rossi). Lang Handbook, p. 94 (1920).

Near DOLGELLEY, with *Aedes vexans*, 27th July, 1923.

Aedes (*Ecculex*) *vexans* (Meig.).

Culex vexans, *Meigen*. Syst. Beschr., Vol. VI, p. 241 (1830).

Ochlerotatus vexans (Meig.). Lang Handbook, p. 85 (1920).

This species has been obtained from one locality; in a field on the right hand of the River Wnion, about half a mile below DOLGELLEY (altitude under 50 feet).

On the evening of 22nd July, 1922, I captured a number of imagines while beating a patch of rushes. On 24th July, a careful search led to the discovery of the larvae, in a small ditch.

In July, 1923, I again visited DOLGELLEY, and found this pool dry. On Monday, 22nd July, the pool was filled as a result of a flood. On Friday, 27th July, I obtained a large number of larvae. An attempt to breed the imagines failed, all the larvae dying, though one pupated. Most of the skins cast were preserved and mounted; a careful examination of this material showed it to contain, besides *Aedes vexans*—in all instar save the first—*Aedes caspius*, *Aedes punctor meigenanus*, *Aedes rusticus*, *Culex pipiens*, and *Theobaldia annulata*.

The imagines, apparently, spend the day among the vegetation. The females attack man.

Apparently, since larvae appear soon after the pool fills with water, it hibernates as an egg; no larvae could be found in the late autumn of 1922.

Culex pipiens, Linn.

Culex pipiens, Linnaeus. Syst. Nat., Ed. X, p. 602 (1758).

This species is everywhere common. It breeds, in my experience, in any type of water, natural or domestic. I have obtained the larvae from streams, ponds, pools in marshes, water in hoof marks, and all sorts of rainwater receptacles. It will breed in very foul situations. It is easy to breed in the laboratory.

The female hibernates in dark, cool cellars or lofts.

It rarely, if ever, attacks man. I have never succeeded in getting it to bite under experimental conditions.

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THE FREQUENCY OF INDICANURIA

BY

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TEST EMPLOYED

To five ccs. of urine in a test tube were added one large drop of five per cent. potassium chlorate, then five ccs. of strong hydrochloric acid, followed by five ccs. of chloroform, the contents being mixed by inverting the closed test tube a couple of times. If no definite blue colouration appeared in the separated chloroform after thirty minutes, the result was regarded as negative.

RESULTS OBTAINED

Three hundred and eighty cases were examined on one occasion each; of these, ninety were apparently healthy, normal individuals, twenty-two (24 per cent.) of whom gave positive results and sixty-eight (76 per cent.) negative. It is to be noted in this connection, that the same individual may give different results on various dates, or even at different hours on the same day. Thus a normal case tested on twenty occasions, was negative on twelve and positive on eight, a negative result frequently alternating with a positive. The remaining two hundred and ninety cases were undergoing treatment for various disorders. The results of the tests are shown in the table on page 550.

Remarks

The presence of indicanuria is usually attributed to excessive putrefactive changes in the intestine (Emerson (1913), Hawk (1919), Cole (1920), Heitzmann (1921)). Its presence in sprue cases is remarked on by several authorities. Thus Cammidge (1912) found it present in eighty-five per cent. of his cases; Rademaker (1906) mentions it as a diagnostic point, while Castellani and Chalmers (1919) and Byam and Archibald (1923) both refer to its presence; Bahr (1915) notes its occurrence in some of his cases, but regards its presence as of no great significance. Three out of

TABLE

Showing the frequency of indicanuria amongst three hundred and eighty individuals

	Number examined	Percentage positive
Normal individuals	90	24
Sprue	4	75
Amoebic Dysentery	35	94
Bacillary Dysentery	5	0
Diseases of stomach and small intestine other than the above ...	6	17
Diseases of large intestine other than the above	23	56
Diseases of the genito-urinary tract	17	30
Surgical conditions other than the above	65	15
Lung cases	62	40
Malaria	20	50
Various	53	27

the four cases examined by the present writer were positive. It will be seen from the table that the highest percentage of positive results (94 per cent.) was obtained from amoebic dysentery patients, while the five bacillary dysentery cases examined were all negative. Obviously the number of bacillary cases examined is too small to allow of definite conclusions being drawn, but the marked disparity between the two would suggest that the test may be of some value for differential diagnosis. Quincke and Roos (1893) have drawn attention to the constant presence of indican in the urine of two amoebic dysentery cases, observed by them for respectively eight and eleven months. Ten out of twenty cases of malaria examined gave positive results. The increase of indican in this disease has already been remarked upon by Marchiafava and Bignami (1900) and Craig (1909).

CONCLUSIONS

Indicanuria occurs in about twenty-five per cent. of apparently normal individuals.

It was present in ninety-four per cent. of amoebic dysentery cases, but was absent from the urine of five cases of bacillary dysentery.

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MISCELLANEA

NOTES ON CESTODE PARASITES FROM A DUCK

On July 5th, 1922, A. W. Noel Pillers, Esq., F.R.C.V.S., obtained from York the intestine of a duck, which was found to contain about 300 Cestode parasites.

As the gut had been removed about two days previously, the parasites were partially moribund. The gut was slit open and placed entire in hot Schaudin's preserving fluid, and later, some hundreds of parasites were removed. They were identified as under:—

(1) *Hymenolepis megalops* (Nitszch, 1829), Par., 1899.
About 20 specimens.

(2) *Hymenolepis coronula* (Duj., 1845), Cohn, 1901.
About half the collection consisted of this species. The rostellum was armed with about twenty hooks, measuring from 13μ to 17μ, of the shape figured by Lühe.

(3) *Aploparaksis furcigera* (Nitzsch, 1819), Fuhrmann, 1908.
Over one hundred specimens were obtained. Microscopical examination of stained specimens revealed the fact that, whilst nearly all segments contained one testis only, other segments contained two, whilst testes were entirely absent from other segments. This phenomenon was noticed in seven or eight strobilae.

T. SOUTHWELL.

NOTES ON PARASITIC WORMS FROM THE GOLD COAST

Dr. J. W. S. Macfie sent a number of specimens from Accra, Gold Coast, West Africa, which were identified as follows:—

(1) *Davainea tetragona* (Molin, 1858), R. Blanchard, 1891.
A large number of specimens from hens.

(2) A coenurus from the jaws of *Mus rattus*, and also from the pleural cavity of *Cricetomys gambianus*.

This larval form was first described by Turner (1919). The adult form is not known; the hooks bear a close resemblance to those of *C. cerebralis* and of *C. serialis*.

T. SOUTHWELL.

CITTOTAENIA LAGORCHESTIS, LEWIS, 1914

This worm was obtained by Dr. Maplestone from the stomach of an agile wallaby (*Macropus agilis*), taken near Townsville, North Queensland.

T. SOUTHWELL.

DAVAINEA LEPTOTRACHELA, HUNG., 1910

A complete cestode worm from the small intestine of *Turdus semitorquata* (Turtle dove), Pietermaritzburg, Natal, was collected and presented to the School by Mr. Hill, Pietermaritzburg. It proved to be a specimen of the above species.

The suckers are armed and the genital pores irregularly alternate; ovary asymmetrical, situated slightly on the pore side; three or four eggs per capsule, the capsules extending in posterior segments to the lateral margins.

Hungerbühler recorded this species from *Pteroclidurus namaquus* (Grouse).

T. SOUTHWELL.

PARAMPHISTOMUM CERVI IN A HORSE

Some worms collected in May, 1920, from a horse at Tamale, Northern Territories, Gold Coast, and kindly sent to us by Dr. K. B. Allan, proved to be *Paramphistomum cervi*. This record is of interest because, so far as we are able to ascertain, this parasite has not previously been obtained from horses, and is not mentioned as occurring in this host by Maplestone in the 'List of Amphistomes arranged under their hosts' appended to his recent revision of the Amphistomata of Mammals.

J. W. S. MACFIE.

A NOTE ON *AUCHMEROMYIA LUTEOLA*, Fab.

The bionomics of this fly and its larva the Congo Floor Maggot, first described by Dutton, Todd and Christy (1904), have been very fully described in recent years by Roubaud (1914).

Fig. 1. *Auchmeromyia luteola.* Larva feeding on human skin. × 10 circ.

The photograph shows the method of feeding which the larva adopts. It stands more or less at right angles to the skin, and has such a firm hold that when the limb is turned over it goes on feeding in a hanging position with equal facility. The feed lasts for as much as an hour in many cases.

The larvae, of which one is photographed feeding on the human arm, were brought alive to England from Sierra Leone at room temperature in sand.

B. Blacklock.

ANCYLOSTOMUM CEYLANICUM IN CATS AND DOGS OF SOUTH INDIA

Ancylostoma caninum and *A. ceylanicum* were found in all of five dogs examined at the Veterinary College, Vepery, Madras; in the single cat examined only *A. ceylanicum* was found.

L. S. Parameswara Ayyar.

THE URINE IN MALARIA

Nephritis as a concomitant of malignant tertian malaria is referred to by most authorities, but its appearance in quartan and simple tertian seems less well known. The following is a record of sixteen consecutive cases of malaria examined at the Liverpool School of Tropical Medicine.

Parasite	Number of cases examined	Number of cases positive
P. malariae	1	1
P. vivax	2	2
P. falciparum	13	7

Protocols of Positive Cases*

Number of case	Duration of attack	Quinine	Temperature	Parasite	Albumin	Deposit in 5 c.c.'s of centrifuged urine
1	4 days	Yes	103°	*P. malariae*	+	A few granular casts and renal cells.
2	3 weeks	Yes	105°	*P. vivax*	+	A few casts and renal cells.
3	3 weeks	Yes	98°	*P. vivax*	o	A few renal and red cells.
4	3 weeks	Yes	98°	*P. falciparum*	+	A few hyaline casts and renal cells.
5	2 weeks	Yes	...	*P. falciparum*	+	Large numbers of granular and hyaline casts and a few renal and red cells.
6	2 months	Yes	98°	*P. falciparum*	+	Nil.
7	2 months	Yes	98°	*P. falciparum*	+	A few renal and red cells.
8	1 month	Yes	103°	*P. falciparum*	o	A few renal and red cells.
9	2 weeks	Yes	98°	*P. falciparum*	o	Many renal cells ; a few red cells.
10	3 weeks	No	98°	*P. falciparum*	+	A few casts and renal cells.

* By 'positive' is meant the occurrence of any, or any combination, of the following, albumin, casts, renal epithelium.

R. M. Gordon.

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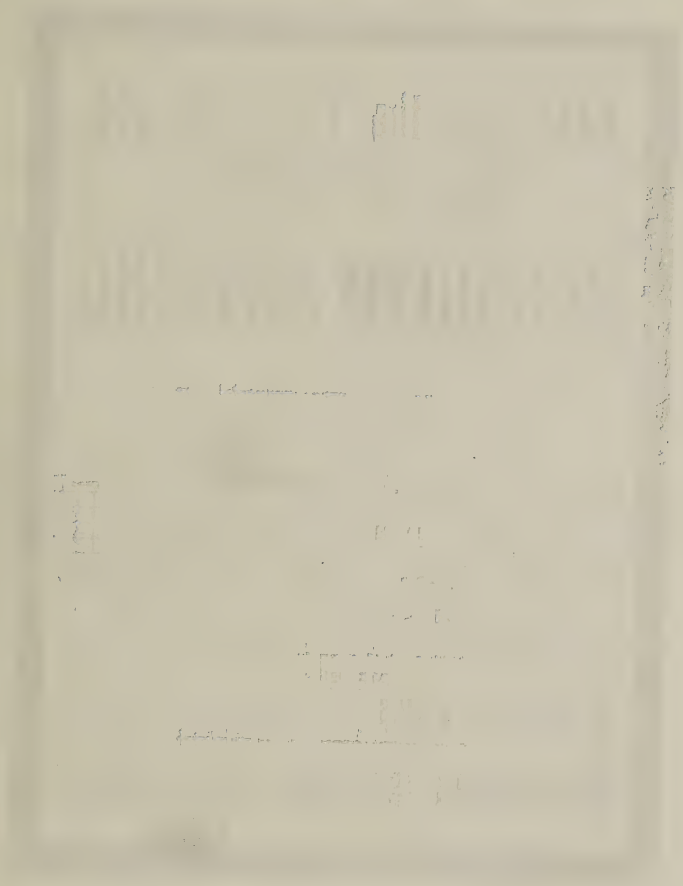

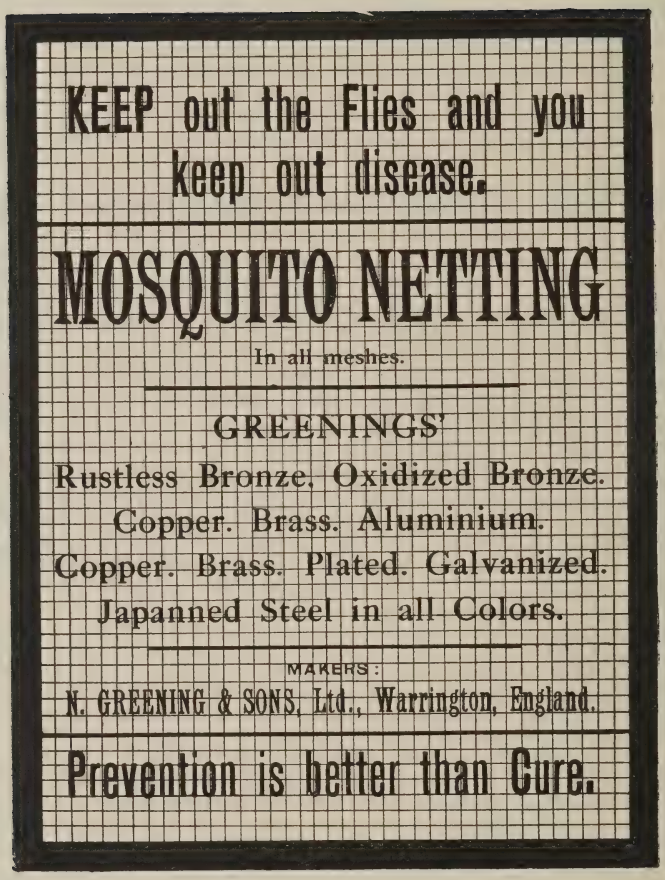
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